# Plaque retention by self-ligating versus elastomeric orthodontic brackets: Quantitative comparison of oral bacteria and detection using ATP-driven bioluminescence

A thesis presented by

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Submitted in partial fulfillment of the requirements for the degree of Master of Science in

Orthodontics Oregon Health and Science University

Portland, Oregon

October 2008

**Plaque retention by self-ligating versus elastomeric orthodontic brackets: Quantitative comparison of oral bacteria and detection using ATP-driven bioluminescence**

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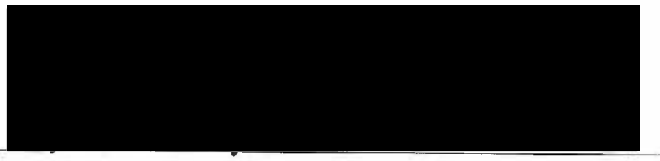

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**Acknowledgements**

I would like to express my gratitude to Dr. Curtis 'Curt' A. Machida as well as Dr. Tom Maier for their guidance throughout the study, especially for their laboratory expertise and vast understanding of research methodology. I thank Dr. David Covell, Jr. for his clinical advice and the Department of Orthodontics for financial support of this project. I would also like to extend my appreciation to the laboratory personnel, Rebecca Sauerwein, Tyler Finlayson, Jennifer Kimmell, Ying Bai, Janna Hickerson and Iraj Kasimi who collectively dedicated literally hundreds of hours in the preparation of samples and for conducting the bulk of the laboratory analysis. I extend a special thanks to all the clinical faculty and co-residents (Drs. Jennifer Crowe, Drew Herion, Tyson Buck) who agreed to help coordinate the treatment of the patients that were enrolled as subjects in this study. Finally, thanks to my wife, Alexis Pellegrini, for support throughout this project.

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## ABSTRACT

**Introduction:** Fixed orthodontic appliances can hinder the maintenance of good oral hygiene, resulting in bacterial plaque accumulation and an increased risk of subsequent enamel decalcification, visible on the facial surfaces of teeth surrounding brackets as un-esthetic white spot lesions. Several studies have investigated the effects of fixed orthodontic appliances on the microbial flora profile, but few have compared the effects of arch wire ligation method, and few studies have managed to obtain a quantitative estimate of the plaque and salivary accumulation that occurs with the bonding of fixed appliances. This is the first study that we are aware of that makes use of the technique of ATP bioluminescence to obtain a quantitative estimate of the changes in microbial levels that occur around fixed appliances, as well as the only study that compares the effects of self-ligation as opposed to a traditional ligation methods. The objectives of this randomized clinical study were to enumerate and compare plaque bacteria surrounding two bracket types, self-ligating (SL) versus elastomeric-ligating (E), and to determine if ATP-driven bioluminescence could be used in the rapid assessment of bacterial load in plaque. **Design**: A randomized, prospective clinical trial employing a split-mouth design. **Material and Methods:** Subjects were 14 individuals (6 males, 8 females; ages 11-17 years) about to start fixed orthodontic treatment. In all patients, the lateral incisor on one side of the maxillary dental arch randomly received a bracket ligated with elastomers while the contra-lateral lateral incisor received a self-ligating bracket. Mandibular lateral incisors received the reverse configuration. All other teeth received self-ligating brackets. In two patients, only upper appliances were placed. Plaque samples were taken from facial surfaces of the lateral incisors, at the periphery of the tooth-bracket interface, on two

occasions: $T_1$ (1 week) and $T_2$ (5 weeks.) Salivary samples were also be taken at baseline $T_0$ (before bonding) and at each time point thereafter. Plaque specimens were assayed for oral bacteria, and subjected to ATP-driven bioluminescence determinations using a luciferin-based assay. Mean, standard deviation, and significance values (p; Student's *t*-test) were calculated. **Results:** In the majority of patients, teeth bonded with SL attachments exhibited fewer bacteria in plaque than teeth bonded with E brackets. At 1 and 5 weeks post-bonding, means for SL versus E brackets were statistically lower for total bacteria and for oral streptococci ($p<0.05$). ATP bioluminescence values were statistically correlated to numbers of total oral bacteria and oral streptococci, and had correlation coefficients of 0.895 and 0.843, respectively. **Conclusions:** The self-ligating appliances promote reduced retention of oral bacteria, and ATP bioluminescence may serve as a useful tool in the rapid quantification of bacterial load and for assessing oral hygiene during orthodontic treatment.

# INTRODUCTION

The presence of acid-producing bacteria, colonizing the tooth surface and surrounding orthodontic appliances, leads to enamel demineralization and often causes alterations in the appearance of the enamel surface (Gorelick,L. 1982; O'Reilly,M.M. 1987; Artun,J. 1986; Geiger,A.M. 1983; Mizrahi,E. 1982). The change in appearance often is an esthetic concern that can persist for many years post-treatment (Ogaard,B. 1989). In addition, decalcification related to bonded orthodontic appliances appears to occur primarily in the immediate proximity of the brackets and not farther away along the facial surface (O'Reilly,M.M. 1987; Gwinnett,A.J. 1979). Thus, the prevention of enamel demineralization during bonded appliance therapy, especially notable and esthetically unsightly at the periphery of brackets, poses a significant challenge to orthodontic professionals.

The recent development of the acid-etch bonding technique has significantly changed the practice of orthodontics. Prior to the introduction of the bonding technique, orthodontic brackets were attached to metal bands that had to be individually fit and cemented to each tooth. Bonded brackets have many advantages over bands because of increased esthetics, ease of placement and removal, and accessibility for oral hygiene (Alexander,S.A. 1991; Boyd,R.L. 1992). Nonetheless, bonded orthodontic brackets impede the maintenance of good oral hygiene, resulting in plaque accumulation and a significantly increased risk for enamel decalcification.

The bonding of fixed orthodontic appliances hinders good oral hygiene, and creates new shelters for microbial colonization. During treatment, there are documented increases in the amounts of the cariogenic microorganisms, *Streptococcus mutans* and lactobacilli, in saliva and

dental plaque of treated individuals (Forsberg,C.M. 1991; Fournier,A. 1998; Corbett,J.A. 1981; Rosenbloom,R.G. 1991). While several studies have investigated the effects of fixed orthodontic appliances on the microbial flora profile, few studies have compared the effects of the bracket architecture, specifically the arch wire ligation method (Forsberg,C.M. 1991; Turkkahraman,H. 2005; Sukontapatipark,W. 2001), or have obtained a quantitative evaluation of the bacterial accumulation that occurs with the bonding of fixed appliances.

Rapid ATP-driven (adenosine triphosphate) bioluminescence assays have long been used as a quantitative measure of microbial numbers, and more recently in dental plaque (Robrish,S.A. 1978; Robrish,S.A. 1979; Ronner,P. 1978, Griffiths,M.W. 1996). Bioluminescence assays measuring energy metabolites, including ATP, have been shown to have high correlations with plaque mass obtained from both humans and animal subjects (Robrish,S.A. 1978; Robrish,S.A. 1979; Ronner,P. 1978, Griffiths,M.W. 1996; Crouch,S.P. 1993).

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

# LITERATURE REVIEW

## Enamel Demineralization

Iatrogenic decalcification of tooth enamel and the development of visible white spot lesions are undesirable and unfortunate sequelae of fixed orthodontic therapy, potentially detracting from the esthetic benefits often achieved through the orthodontic correction of a malocclusion. Visible decalcification related to fixed orthodontic therapy has also led to litigation, with courts and juries placing liability on orthodontic practitioners (Machen,D.E, 1991). The development of the acid-etch bonding technique (Buonocore,M.G. 1955) and the subsequent orthodontic application via the bonding of brackets in lieu of full banded appliances (Newman,G.V. 1964), has not only facilitated the efficiency of orthodontic appliance construction, but also reduced the amount of tooth surface area covered with appliances. Nonetheless, bonded orthodontic brackets hinder the maintenance of good oral hygiene, resulting in bacterial plaque accumulation. Dietary consumption of fermentable carbohydrates and the presence of bacterial plaque on the tooth substrate are the prerequisites for demineralization of tooth enamel (Mitchell,L. 1992; Zachrisson,B.U. 1971). Acid-producing bacteria colonizing the tooth surface surrounding orthodontic appliances leads to enamel demineralization, often causing an alteration in the appearance of the enamel surface(Gorelick,L. 1982; O'Reilly,M.M. 1987; Artun,J. 1986; Geiger,A.M. 1983; Mizrahi,E. 1982; Ogaard,B. 1989; Gwinnett,A.J. 1979; Forsberg,C.M. 1991; Rosenbloom,R.G. 1991; Huser,M.C, 1990).

**Enamel Demineralization:** ***Prevalence and distribution***

The prevalence of white spot lesions among patients who have undergone fixed orthodontic treatment has been reported by several authors, with varying results. Gorelick et al. (1982) report 50 percent in treated versus 24 percent for non-treatment control patients. Artun et al. (1986) report 59 percent, Geiger et al. (1983) report 34 percent, and Banks et al (1994) report 75 percent prevalence, with differences partially accounted for by variations in decalcification assessment, research methodology, oral hygiene, diet and other patient differences. Despite a rather wide spectrum of prevalence, the development and appearance of unsightly white spot lesions during the course of fixed appliance therapy consistently presents a significant problem to orthodontists.

With regard to the distribution of affected teeth, Gorelick et al. (1982) and Mizrahi (1983) found maxillary incisors and first molars to be the teeth with the highest prevalence. In fact, Gorelick et al. (1982) found that the teeth with the highest individual incidence were maxillary lateral incisors, with 23 percent of teeth being affected, with the second most commonly affected tooth being the maxillary centrals, with three times less at 8.4 percent. Interestingly, the same researchers found the length of treatment to have no effect, with patient in treatment for 12-16 months experiencing the same incidence of white spot lesions as those involved in longer treatment schedules (up to 36 months.)

While a protracted treatment course may play a less-than intuitive role in the development of white spots, histological studies have demonstrated the rapidity with which decalcification can occur. In a scanning electron microscopic histological study, Sukontapatipark et al. (2001) found the area surrounding the bracket base was almost completely covered with a

thick accumulation of bacteria within one week after the placement of appliances. The authors attributed this to excess composite, with adjacent smooth areas exhibiting a less mature monolayer of bacteria. In a similar manner, Glatz, et al. (1985) and O' Reilly et al. (1987) demonstrated that measurable histologic decalcification (up to 15% demineralization, to a depth of 50-75μm) occurred around orthodontic appliances after only a month of placement. Furthermore, O'Reilly et al. found that decalcification related to bonded orthodontic appliances occurred most immediately around the appliance and not farther away along the facial surface. Thus, the prevention of demineralization during bonded appliance therapy, especially notable and esthetically unsightly at the periphery of brackets, poses a challenge to orthodontists.

**Enamel Demineralization: *Prevention***

**Adhesive Systems**

Given these potential side effects, several approaches have been recommended to help prevent the accumulation of plaque bacteria and subsequent enamel damage around fixed appliances. Adhesive systems that possess antibacterial effects or inherent re-mineralization properties are currently available, and there have been numerous reports on the properties of these materials (Ortendahl,T. 1997; Millett,D.T. 1996; Mitchell,L. 1992; Banks,P.A. 1997; Hallgren,A. 1993) Glass ionomer cements (GIC) , which have the ability to release and reabsorb fluoride (Silverman,E. 1995) and fluoride-releasing composites have attracted attention because of the aforementioned properties. Hallgren, et al. (1993) found significantly lower levels of mutans streptococci in plaque isolated from GIC-retained brackets compared to those bonded with composite resins, after one month of appliance placement. However, Ortendahl et al. (1997) demonstrated that longer-term clinical effects (mean treatment time of 9.5 months) were not

statistically significant, although the mean levels of streptococci in plaque isolates surrounding GIC-retained brackets were less. This may suggest that the beneficial effects of fluoride-releasing glass ionomer cements are ephemeral, and taper off as treatment time progresses. Furthermore, glass ionomer cements have demonstrated unfavorable bonding performance, such as inadequate bond strengths for effective clinical use (Ortendahl,T. 1997; Millet,D.T. 1996; Cook,P.A. 1988) In a review of orthodontic bonding with glass ionomer cement, Millet et al (1996) indicates that the consensus of published literature germane to clinical performance suggests that conventional glass ionomer cements are unreliable for clinical orthodontic bonding.

Fluoride-releasing orthodontic bonding composite materials, while performing better than GIC in terms of reduced bond failures, unfortunately, provide less fluoride release than glass ionomers over shorter periods of time, and have been shown in long-term clinical trials to be ineffective in preventing enamel decalcification (Banks,P.A. 1997; Mitchell,L. 1992).

**Sealants**

'Sealants' (the intermediary liquid layer applied to and subsequently cured) that are placed on the acid-conditioned enamel prior to composite-retained bracket placement have been studied in relation to orthodontic delcalcification. Numerous names for this layer have appeared in the literature: sealant, primer, adhesive, bonding resin, unfilled resin, low viscosity resin as well as numerous proprietary terms. The terms are used interchangeably, and are of various compositions. Initially, these sealants were chemically cured and were believed to provide a measure of protection to the acid-etched enamel, as well as protection against demineralization (Zachrisson,B.U 1975). Banks and Richmond (1994) conducted a clinical study in which 80 individuals undergoing fixed appliance treatment were divided into two groups of 40. One group

was treated with a viscous chemical cure sealant/bonding system (Maximum Cure®) and the other group was treated with a non-viscous light-cured sealant/bonding system (Transbond VLC ®). Alternate teeth, where no sealant was placed, served as controls. In the Maximum Cure® (viscous chemical cured sealant) group, 73 % of patients experienced decalcification—In terms of individual teeth, 31% of the control teeth and only 19% of the sealed teeth were effected with decalcification. This 12% difference was statistically significant. The Transbond VLC ® group showed a similar 75% decalcification rate among patients, but there were no significant differences between sealed (23%) and control (25%) teeth. Thus, sealants applied to enamel surfaces have demonstrated the potential for small benefit (Banks,P.A. 1994) but are technique sensitive and of poor durability and may indeed increase the risk of decalcification as a result of low abrasion resistance (Ceen,R.F. 1980).

**Fluoride Supplementation**

It has been suggested that if adequate preventive care (good oral hygiene, fluoride supplementation, and professional care) is practiced and maintained during the course of orthodontic treatment, then decalcification rates may be reduced. Home care regimens such as daily NaF (sodium fluoride) mouth rinses have shown some significant measure of protection as well, but suffer from the potential for non-compliance as found in many home-care programs. (O'Reilly,M.M. 1987; Benson,P.E. 2004; Zachrisson,B.U. 1975, Geiger,A.M. 1992) Geiger et al. (1992) conducted a clinical study to determine if a twice-daily self-administered rinse of 10 mL of (0.05%) NaF (sodium fluoride) would influence white spot lesion formation during orthodontic treatment. It was concluded that a significant reduction in enamel white spot lesions can be achieved during orthodontic therapy through the use of a 10 ml neutral sodium fluoride

rinse. The more closely patients complied with the prescribed use, the more likely they could expect a decrease in the occurrence of lesions. However, only 13% of the 206 enrolled patients fully complied with the rinse protocol. The inability to achieve adequate compliance even within the confines of a well-controlled clinical trial serves to highlight the complexities associated with patient oral hygiene motivation and compliance. The effectiveness of such supplemental home-care preventive regimens requires *additional* cooperation on the part of patients who (outside of a well-controlled clinical study) unfortunately, may be those at highest risk, exercising less-than-adequate home care in the first instance.

**Fluoridated Elastomerics**

Recently, fluoride-containing elastomeric modules, which circumvent the compliance problem, have been suggested for reducing plaque accumulation and decalcification. The effects of fluoridated elastomers on plaque reduction and decalcification risk around fixed appliances have been investigated, and while some studies indicate a beneficial effect (Banks,P.A. 2000; Mattick,C.R. 2001), other results show an insignificant effect (Benson,P.E. 2004; Miura,K.K. 2007). Banks, et al (2000) conducted a controlled clinical trial to evaluate the effectiveness of stannous fluoride-releasing elastomeric modules (Fluor-I-Ties) and chain (Fluor-I-Chain) in the prevention of enamel decalcification during fixed appliance therapy. Forty-nine patients (782 teeth) were included in the experimental group (received fluoridated elastomers), and forty-five patients (740 teeth) were in the control group (received non fluoride-releasing elastomers.), with the same type of elastomers being replaced at each visit. Enamel decalcification incidence and distribution were recorded using an index by direct clinical observation. In the control group enamel decalcification occurred in 73 per cent of patients and in 26 per cent of all teeth. In the

experimental group the corresponding incidence was 63 and 16 per cent, respectively. The overall reduction in score per tooth produced by the fluoride-releasing elastomerics was 49 per cent, a highly significant difference ($P < 0.001$). A significant difference was seen in all but the occlusal enamel zones. The majority (over 50 per cent) of lesions occurred gingivally. The teeth most severely affected were the maxillary lateral incisors and mandibular second premolars. There was no difference in treatment duration between groups.

Mattick et al. (2001) also conducted a controlled clinical trial to study the effects of fluoride releasing elastomeric modules on the incidence of decalcification around orthodontic brackets during a complete course of orthodontic treatment, but chose to instead utilize a split mouth design, rather than separate groups. Twenty-one (21) consecutive patients (126 teeth) were included, where one side (left or right) was randomly assigned to the experimental group, and the opposite side served as a control throughout their course of orthodontic treatment. Standardized photographs were taken of the upper labial segment before and after completion of orthodontic treatment, and the degree of decalcification assessed in each tooth quadrant, using a modification of the Enamel Defect Score. The results were that decalcification was found to occur in both treatment groups, though to a significantly greater degree on the control side ($p = 0.002$). The fluoride module side showed significantly fewer serious decalcified lesions than the control ($p = 0.013$). Both this study and that of Banks et al. (2000) suggest that the use of fluoride releasing elastomeric modules reduces the degree of decalcification experienced during orthodontic treatment.

Two different studies, however, found that fluoridated elastomers were not effective in reducing the potential for orthodontic related decalcification (Benson,P.E. 2004; Miura,K.K.

2007). Benson et al. (2004) conducted a randomized clinical trial, employing a split mouth, crossover design to investigate the effect of fluoridated elastomers on the quantity of disclosed dental plaque surrounding orthodontic brackets. The subjects were 30 individuals about to start fixed orthodontic treatment. The study consisted of two experimental periods of 6 weeks with a washout period between. Fluoridated elastomers were randomly assigned at the first visit to be placed around brackets on specific teeth, with non-fluoridated elastomers were placed on the contra-lateral teeth. After 6 weeks (visit 2) the elastomers were removed, the teeth disclosed and a photograph taken. Non-fluoridated elastomers were placed on all brackets for one visit to allow for a washout period. At visit 3, fluoridated elastomers were placed on the contra-lateral teeth to visit 1. At visit 4, the procedures at visit 2 were repeated. The photographs were scanned and the area and proportion of the buccal surface covered with disclosed plaque was measured using computerized image analysis. The authors concluded that fluoridated elastomers did not affect the quantity of disclosed plaque around the orthodontic brackets. In a systematic review conducted the following year, Benson et al. (2005) reports the deficiencies in study design, such as lack of randomization, unclear or poorly-defined inclusion criteria, or lack of blinding in studies reporting the benefits of fluoridated elastomers. The review reported that there was too high a risk of experimental bias to conclusively indicate a positive effect of fluoridated elastomers on the reduction of white spot lesions. Furthermore, the split-mouth design employed by Mattick et al. (2001) may not be appropriate for studying the effects of fluoride release.

Miura et al. (2007) also studied the effect of fluoridated elastomers but chose not to employ a split mouth design, favoring instead dividing the subjects into separate control and experimental groups. At seven, 14 and 28 days after placement of the elastomeric ligature ties, saliva and plaque surrounding the orthodontic appliance were collected for microbiologic

analysis. The results of this study showed that this material did not cause any statistically significant change in the numbers of *Streptococcus mutans* in saliva or plaque. It would seem that further, well-controlled, randomized studies investigating the effects of fluoridated elastomers will be necessary to evaluate the effectiveness of this modality. Table 1 summarizes and categorizes the compliance required from the patient and effectiveness of various proposed methods to reduce the risk of orthodontic decalcification.

**Table 1: Hierarchy of Compliance: Orthodontic Decalcification**

| Method/ Technique | Pt. Compliance Required (+ = NO, - = Yes) | Operator/ Staff Compliance Required (+ = NO, - = YES) | Effectiveness/ Demonstrated clinical benefit |
|---|---|---|---|
| Appliance itself | +++ | +++ | ? |
| Luting Agent/ cement | +++ | +++ | +/- |
| Fluoridated Ligature | ++ | + | +/- |
| Sealant | ++ | + | +/- |
| External agent:<br>1-Fluoridated mouth rinses<br>2-Fluoride varnises<br>3-OH Improvment/ dentrifice/Rx | 1) ---<br>2)+++<br>3)--- | 1)-<br>2)---<br>3)--- | +++ |
| | | | |

The bonding of fixed orthodontic appliances hinders good oral hygiene, and creates new shelters for microbial colonization. During treatment, there are demonstrated increases in the amounts of *Streptococcus mutans* and Lactobacilli in the saliva and dental plaque (Forsberg,C.M. 1991; Rosenbloom,R.G. 1991). A number of studies have evaluated the effect of fixed orthodontic appliances on microbial flora and periodontal status, but few studies have evaluated the manner of ligation as an additional factor (Sukontapatipark,W. 2001; Turkkahraman,H. 2005; Forsberg,C.M. 1991). However, two of the more recent of these studies were not clinically well-controlled due to a lack of randomization into a split-mouth design

(Turkkahraman,H. 2005; Sukontapatipark,W. 2001)or lacking arch wire engagement because of the fact that only one tooth was bonded in each quadrant(Sukontapatipark,W. 2001). Furthermore, no published studies were found that compared the difference in bacterial colonization profiles between traditional versus that of self-ligation methods. In an effort to quantify the level of microbial colonization immediately surrounding the different appliances, a novel technique (ATP bioluminescence), in parallel with standard media plating, with a standardized sampling protocol was utilized.

**ATP-Driven Bioluminescence**

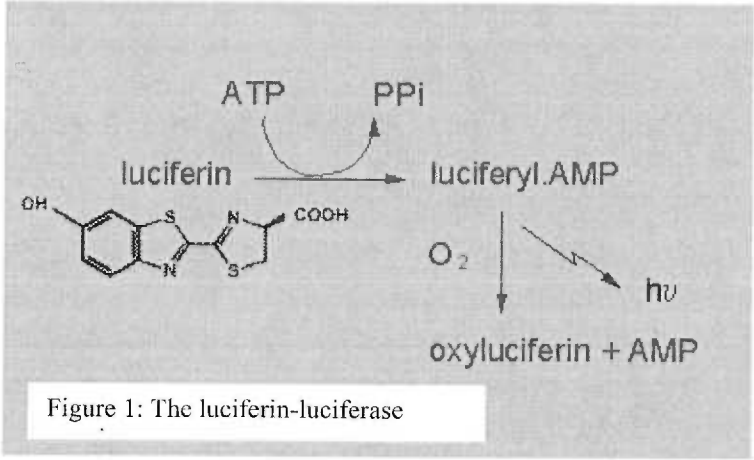


Figure 1: The luciferin-luciferase

The method of adenosine triphosphate (ATP) bioluminescence for the quantification of live bacterial cells is based on the reaction between bacterial ATP using the enzyme luciferase and the cofactor luciferin. (Figure 1) Hydrolysis of ATP by luciferase emits yellow-green light that is detected using a luminometer and reported as a relative light unit (RLU). The intensity of light emitted from the reaction is proportional to the amount of ATP in the sample. In general, the level of ATP in live bacterial cells is relatively constant at given conditions. Thus, the

amount of light emitted during the reaction is proportional to the number of bacteria from which the ATP was released (Griffiths,M.W. 1996). The method of ATP bioluminescence has been demonstrated to have a linear relationship between cell number and measured luminescence using the luciferin-luciferase reaction. (Crouch,S.P 1993; Robrish,S.A. 1978). Furthermore, the sensitivity of the bioluminescence assay facilitates the examination of small dental plaque samples obtained from local sites on tooth surfaces (Robrish,S.A. 1978). The ATP may be quantified in the femtomole range, which is the sensitivity required if samples are to be assayed from individual teeth(Robrish,S.A. 1979). The ATP bioluminescence test (Fig. 2a) Cariscreen® test (Oral BioTech, Albany, OR) has recently been introduced into dental offices as a semi-quantitative means of assessing caries susceptibility, as have several other methods of assessing overall risk for caries development (Fontana,M. 2006). In the Cariscreen® test, a swab is used to sample plaque from the surface of several teeth, followed by immediate lysis of the plaque bacteria and measurement of their ATP. This rapid semi-quantitative assay of plaque bacteria has several advantages over the more time-consuming standard selective plating assays, including rapidity, facility, and possible chair-side application. Another system that can be utilized and available for rapid ATP Bioluminescence analysis is the bench top luminometer with 96 wells (Figure 2b) Veritas® Microplate luminometer (Turner Biosystems; San Diego, CA)

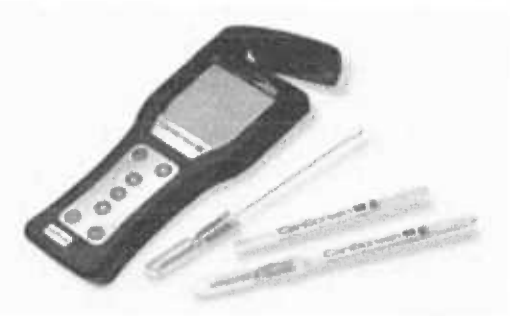


Figure 2a: Cariscreen® (Oral BioTech) Caries Susceptibility Testing Meter and Swabs.

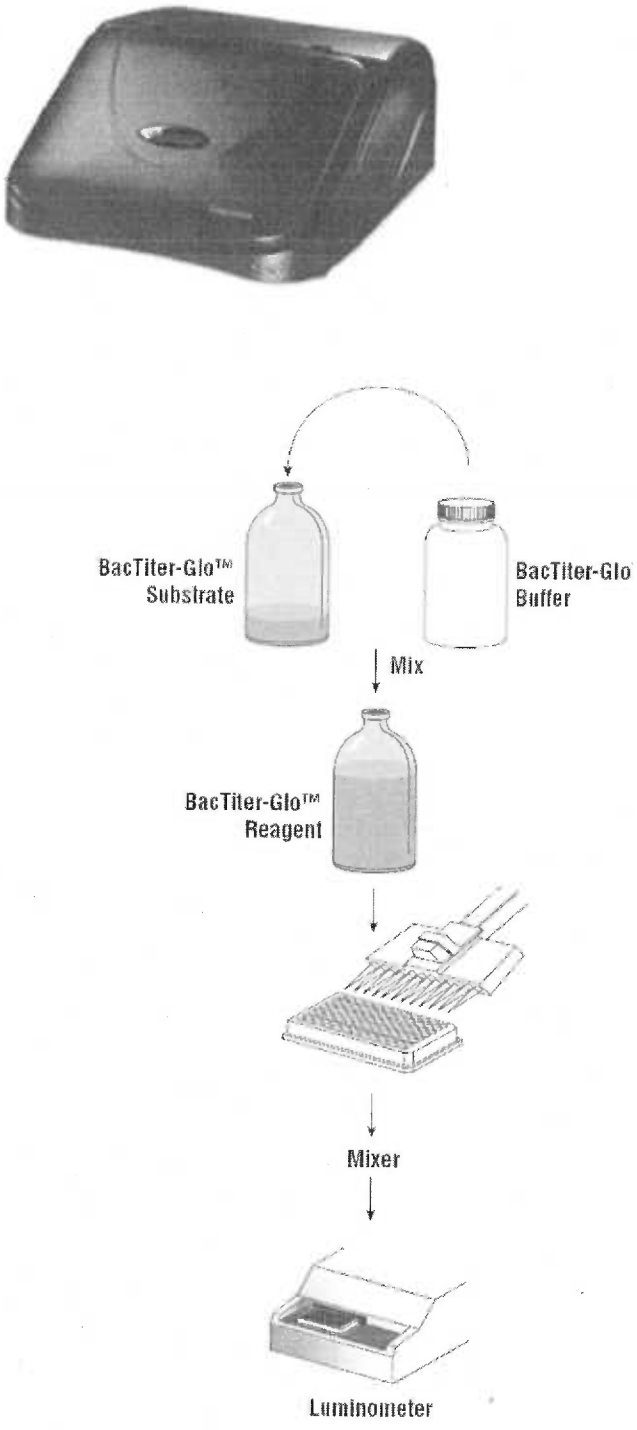



Figure 2b: Veritas® Benchtop luminometer, and schematic cartoon illustration.

The purposes of this randomized, prospective, longitudinal controlled clinical study utilizing a "four quadrant," split-mouth design are: (1) To measure and compare the numbers and types of bacteria found in plaque surrounding two distinct orthodontic brackets, self-ligating versus elastomeric as well as to determine the validity of the method of ATP bioluminescence for the rapid assessment and quantification of plaque bacterial load on tooth surfaces surrounding orthodontic appliances.

## MATERIAL AND METHOD

### *Test Subjects*:

Fourteen patients (12 with full appliances, 2 with appliances on the maxillary arch only) successfully completed the study out of the eighteen (18) patients originally-enrolled, with four patients being excluded because of failure to adhere to the selection criteria or failure to keep one or more appointments for specimen collection. The criteria for inclusion in this study were age, with participants being at dental age 12 or older ($x$ = 13.9 years, range 11.7- 17.2 years) and demonstrated ability to maintain adequate oral health. All patients selected were diagnosed as requiring fixed appliance orthodontic therapy and scheduled for treatment in the Orthodontic Clinic at the Oregon Health & Sciences University (OHSU). Patients who were pregnant, diabetic, using any mouth rinses or interacting medications, including antibiotic therapy within the three months prior to the study, were excluded from participation. Participants were assigned study identifier numbers that were accessible only to the study investigators and were kept in a notebook locked in the Department of Orthodontics.

***IRB and Human Subjects Consent:***

This study was reviewed and approved by the OHSU Institutional Review Board (IRB). In addition to the consent form for routine orthodontic care currently in use in the OHSU Orthodontic Clinic, the parent/guardian of each subject selected for participation was given a second consent form specifically relating to the clinical study. We requested that participants also refrain from eating or drinking one hour prior to the sampling appointments. 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. Signed consent forms specifically for the research study were obtained at that time. We used the OHSU Short Form for non-English speakers and obtained interpreters, as necessary, through the University or the School of Dentistry. A study investigator, interpreter, participant, and the participant's parent/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. The participant's parent/guardian signed the OHSU Short Form, the investigator signed the Written Summary, and the interpreter signed both the OHSU Short Form and the Written Summary. The study called for no additional treatment or procedures not normally performed in the routine oral care provided during initial bonding or orthodontic adjustment 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 was done to ensure that participants initially included in the study that subsequently took an interacting medication would

be 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 and recored on the Data Collection Sheet (Figure 3)

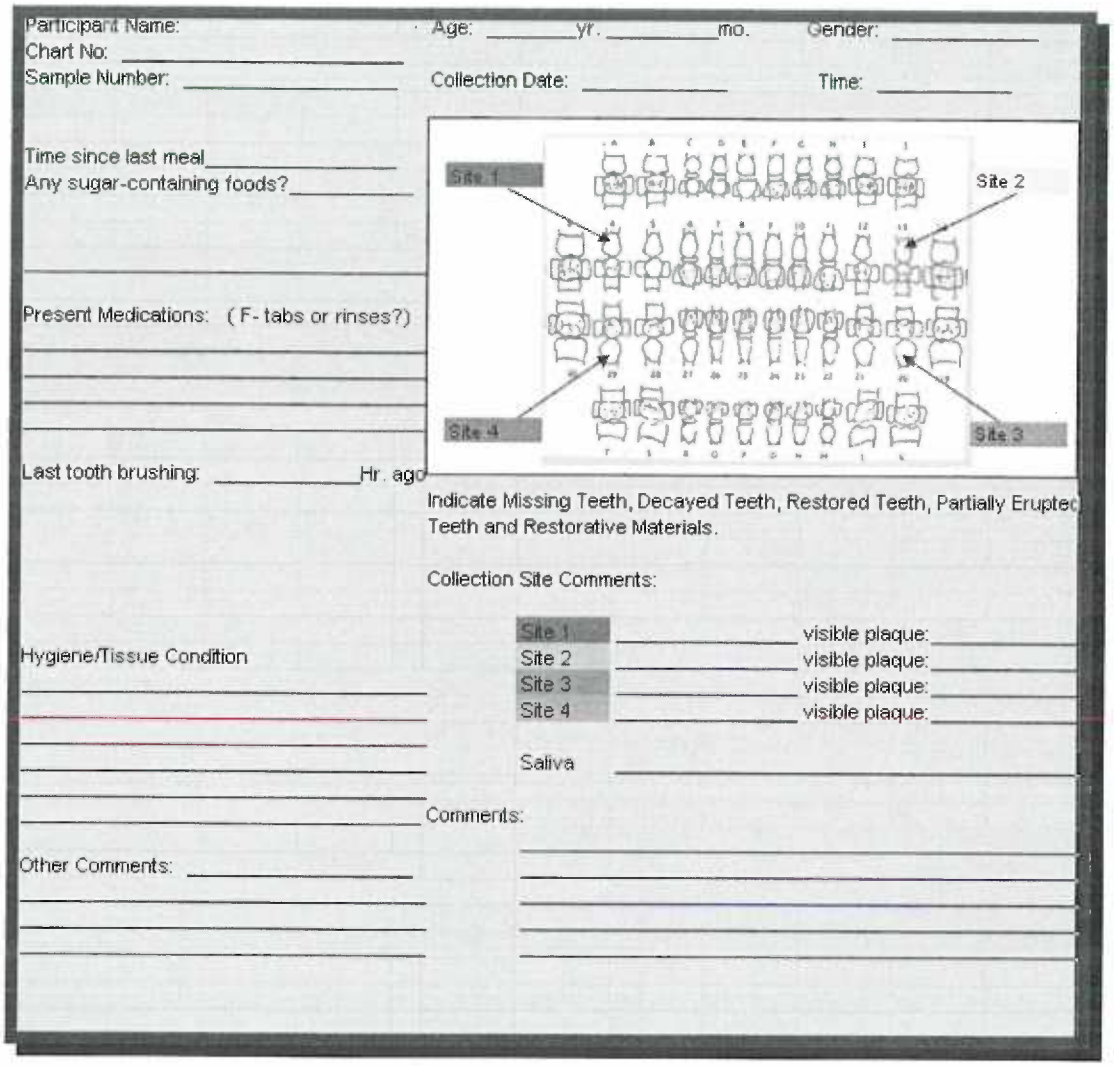



Figure 3 The Data Collection sheet used at each visit.

***Description of Randomized Split-Mouth Design and Subject Preparation*:**

The doctor assigned for the plaque collection (Peter Pellegrini, DDS) utilized a standardized collection technique, 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 hand piece. Patients were given oral hygiene instruction and fluoridated toothpaste and toothbrush, and asked to refrain from other oral hygiene supplements for the duration of the study. At the initial visit, one half of each arch, either left or right sides, was randomly assigned to receive the experimental bracket with the opposite side serving as the control (Figure 4).

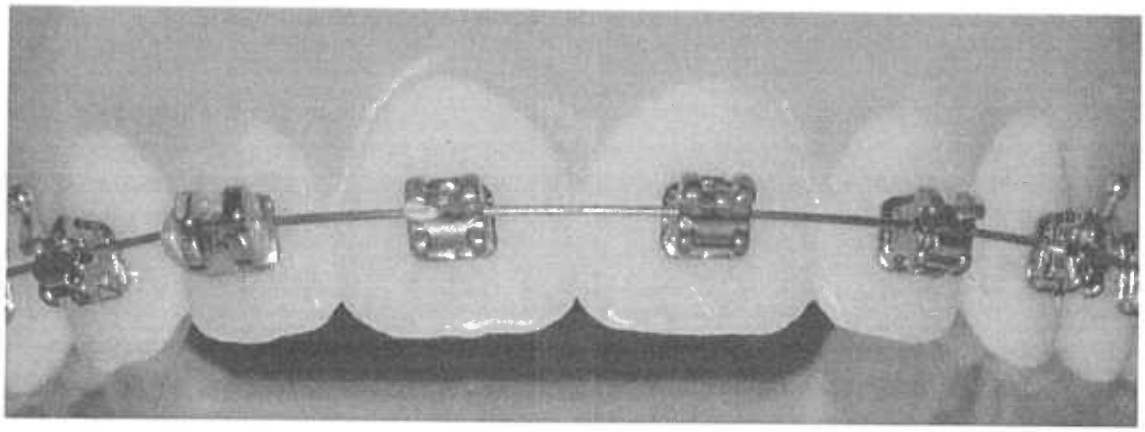


Figure 4: Demonstration model illustrating the randomized allocation of brackets

Figure 5a is a photograph of a left-handed, male adolescent patient who was enrolled in the study, taken approximately 4 months after appliances were bonded. Note the heavy plaque accumulation the gingival inflammation, especially around the patient's right lateral incisor. Note that the patient, in this case, randomly received the same bracket configuration as the model arch illustrated in Figure 4. Specifically, for each arch, the left or right lateral incisors randomly received either the experimental "self-ligating bracket" (0.022"; In-Ovation-R®, GAC International, Bohemia, NY [Figure 5B]) or the control "elastomeric-ligating bracket" (0.022"; Mini-Ovation®, GAC [Figure 5C]; the latter brackets were ligated with silver-colored elastomeric ligatues (AlastiKs®, 3M Unitek, Monrovia, CA). The appliances were directly

bonded using composite resin (Transbond®, 3M Unitek, Monrovia, CA), with all but the lateral incisors bonded with self-ligating brackets (0.022"; In-Ovation-R®).

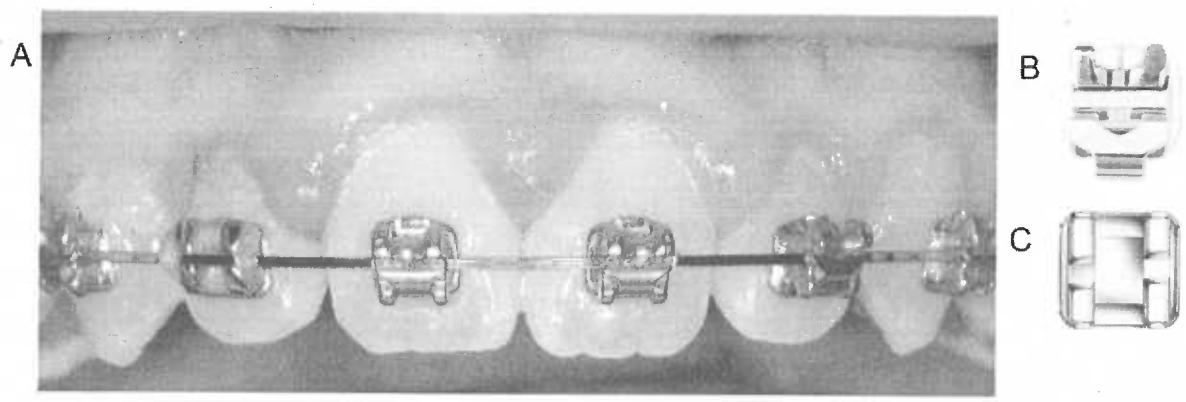



**Figure 5: Experimental design and description of self-ligating and elastomeric appliances. A.** Experimental design on an enrolled patient's maxillary arch; **B.** Self-ligating bracket, Innovation-R,® (GAC International, Bohemia, NY); **C.** Traditional elastomeric-ligating bracket, Mini-Ovation,® (GAC).

***Procedures for Collection of Plaque:***

The sampling investigators utilized a standardized protocol to collect specimens: At each designated sampling visit, the operator carefully removed/disengaged the elastomeric tie/ligation mechanism, and removed the archwires. Plaque specimens were collected from the labial surfaces immediately surrounding the orthodontic brackets of the maxillary and mandibular lateral incisors utilizing a sterilized dental scaler with the same tip dimensions (# 8/9 Orban DE hoe scaler, Hu-Friedy, Chicago, Illinois [Figure 6]). Given the area of increased decalcification generally occurring immediately adjacent to the brackets, a four-pass technique was utilized to move the instrument tip around the circumference of the bracket at the bracket-tooth interface (Figure 7). Four passes, one along the tooth at the bracket interface at the gingival, mesial, distal and occlusal, were used to avoid overloading the instrument tip. All of the specimens from each tooth (left and right incisor for both maxillary and mandibular arch) were placed into four

individual tubes that had anonymous coding, and sealed for transport to the laboratory. The coding of specimens assured blinding of laboratory personnel and helped minimize experimental bias.

**Figure 6: The instrument used for specimen collection**: The # 8/9 Orban® DE hoe scaler (Hu-Friedy, Chicago, Illinois) has a 90° , 1.5 mm tip dimension for standardized and consistent specimen collection.

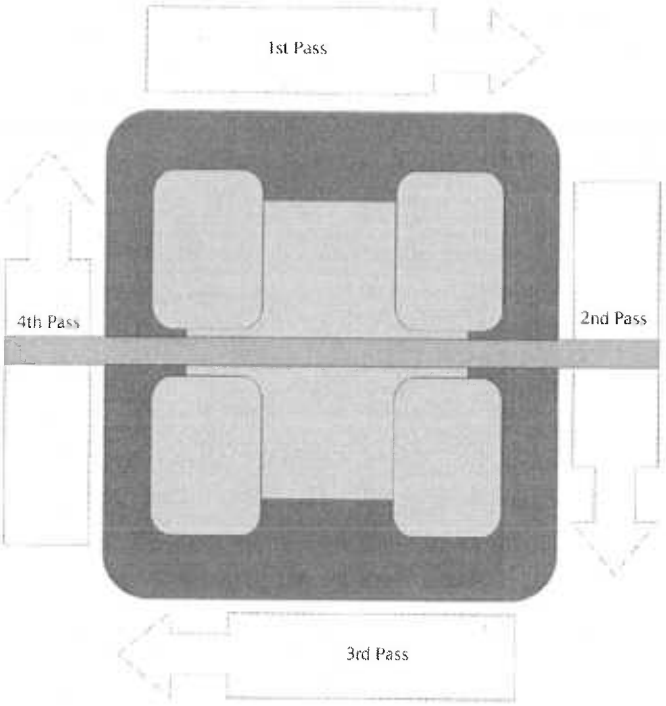



**Figure 7: Four-pass sampling technique.** A standardized, sterilized instrument tip is moved circumferentially around the bracket. Wire is pictured, and the ligation mechanism is carefully removed prior to sampling.

***Saliva Collection Procedures:***

Participants were given a chewing gum-shaped paraffin wax tablet, and instructed to chew the tablet for 1-5 minutes or until several ml of saliva was collected and expelled into a sterile calibrated collection container. The sample was coded with the unique participant

identifier, and transferred to the laboratory for evaluation. At the $T_0$ bonding appointment, collection of saliva occurred prior to placement of appliances.

***Microbiological Analyses of Specimens:***

Five specimens (4 plaque plus stimulated saliva specimens) were collected per subject at each appointment after bonding ($T_1$ and $T_2$: 1 week and 5 weeks after bonding, respectively), with the exception of the bonding appointment ($T_0$ ) where only saliva specimens were collected. Each plaque specimen was diluted in 1 ml of phosphate buffered saline (PBS), in the presence of glass beads, and dispersed by vigorous agitation on a rocker platform (37°C, 10 minutes). Dispersed plaque samples were then subjected to 10-fold serial dilutions in PBS and then plated on enriched blood agar (PML Microbiologicals, Wilsonville, OR) to determine total bacterial numbers. Total oral streptococci numbers were determined by limiting dilution plating on mitis salivarius agar (MSA; Difco™; Becton, Dickinson and Company, Sparks, MD), which utilizes high sucrose and vital dyes as selective agents. MSA will select for numerous strains of oral streptococci with varying abilities to generate acid and effects on decalcification potential. All platings were conducted in quadruplicate, and plates exhibiting colony numbers between 50 and 500 were counted and averaged to determine mean values.

***ATP-Driven Bioluminescence of Specimens:***

Using the luciferin substrate and luciferase enzyme, bacterial ATP can be quantitated by measuring the release of visible light (Ronner,P. 1999). ATP contained in bacteria from plaque specimens was determined with the use of the BacTiter Glo Microbial Cell Viability Assay kit (Promega, Madison, WI; product number G8231), with ATP-driven bioluminescence measured

by the Veritas® Microplate luminometer (Turner Biosystems; San Diego, CA). 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 or absorbance at 600 nM wavelength measured with Novaspec II Visible spectrophotometer). The Veritas® luminometer has a 5-fold dynamic range in RLU readouts.

***Statistical Analysis:***

Using data from two previous studies (Banks,P.A. 2000; Corbett,J.A. 1981}, it was calculated that a sample size of 30 would be sufficient to detect a difference in *S. mutans* count of 30% to a power of 0.85 at a significance level of $\alpha$=0.05. These studies evaluated the upper arch only. In our study, both arches were be evaluated, thus effectively doubling our sample size and power level. Nonetheless, our goal was to have up to 20 patients participate in this study to increase the power and also to account for dropouts in the event that participants fail to meet or continue to adhere to the selection criteria. Eighteen patients were eventually enrolled (Table II). Fourteen patients completed the study, with twelve in full appliances, and 2 receiving maxillary appliances only. Four patients were excluded due to failure to make a sampling appointment, or failure to continue to meet the criteria for inclusion. Descriptive statistics, including the determination of means values for bacterial counts, and corresponding standard deviations were calculated. The mean bacterial counts and ATP-driven bioluminescence determinations (in RLUs) from teeth bonded with SL and E brackets were tested for significant differences using paired *t*-tests (one-tailed, with p values $< 0.05$ considered statistically significant). Based on the results of the bacterial counts and ATP-driven bioluminescence determinations for each of the plaque samples from lateral incisors, contrasting bracket pairs within each arch were assigned to one of the following groups: 1) Those exhibiting the highest number of bacterial counts or RLUs

on the tooth bonded with a SL bracket (SL> E) and 2) Those exhibiting the highest number of bacterial counts or RLUs on the tooth bonded with an E bracket (E>SL). The $chi^2$-test and distribution were used to establish whether a significant difference existed between the numbers of contrasts in each category (Forsberg,C.M. 1991).

**Analyses of specimens collected from participants (Figure 8)**

Five specimens will be collected, per subject, at each time point, ($T_1$ -1-week after bonding, and ($T_2$ -5 weeks after bonding) except $T_0$-bonding appointment-where solely a saliva sample will be taken): Four ('1-4' in Figure 8) quantitative samples from the bracket periphery of each of the 4 sampling sites and one sample of undiluted, stimulated, whole saliva ('5' in Figure 8)

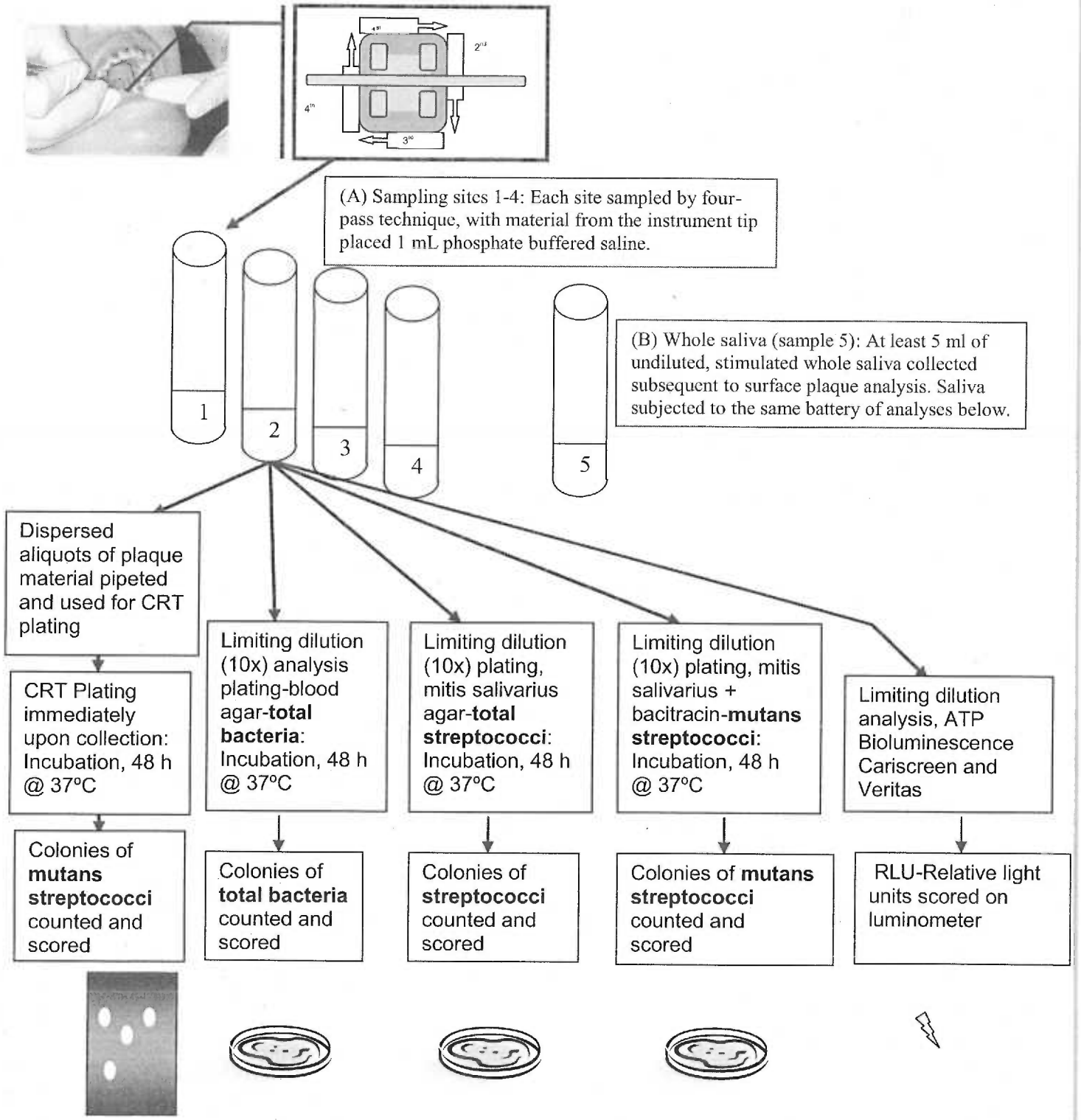



Figure 8: Flow diagram of specimen collection and analysis

## RESULTS

### *Patient Demographics and Positions of Appliances Used in the Study:*

Table 1 describes the patient population in this study, including the randomized allocation of brackets bonded to the lateral incisors. Note that seven patients had E brackets placed on the maxillary right quadrant and SL brackets placed on the maxillary left quadrant, and that seven patients had SL brackets placed on the maxillary left quadrant and E brackets placed on the maxillary right quadrant. Also, in all cases for any given patient, the appliance type was switched in its left-right orientation when appliances were placed in the mandibular arch. Ten individuals were right handed, three individuals were left handed, and one was ambidextrous. Plaque was collected from lateral incisors in the maxillary right quadrant (tooth 7), maxillary left quadrant (tooth 10), mandibular left quadrant (tooth 23), and mandibular right quadrant (tooth 26) (also defined as upper right (UR), upper left (UL), lower left (LL) and lower right (LR) quadrants, respectively, in Table 2).

**Table 1: Patient Demographics and Placement of Brackets on Selected teeth**

| Patient #[1] | Gender | Age | Handedness | Oral Health | Average Time (hrs) brushing before visits[2] | Orthodontic Treatment[3] | (UR) Tooth 7 Bracket Type[4] | (UL) Tooth 10 Bracket Type[4] | (LL) Tooth 23 Bracket Type[4] | (LR) Tooth 26 Bracket Type[4] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 14.6 | Right | good | 1 | full | E | SL | E | SL |
| 2 | M | 14.11 | Right | good | 1 | full | E | SL | E | SL |
| 3 | F | 16.1 | Right | good | 1 | full | SL | E | SL | E |
| 4 | F | 14 | Right | good | 3 | full | SL | E | SL | E |
| 5 | F | 17.2 | Ambidextrous | good | 1 | upper | E | SL | N/A | N/A |
| 6 | F | 12.2 | Right | good | 1 | full | SL | E | SL | E |
| 7 | F | 12.9 | Right | good | 1 | full | E | SL | E | SL |
| 8 | F | 15.9 | Right | good | 2 | full | E | SL | E | SL |
| 12 | F | 12.11 | Left | good | N/A | full | E | SL | E | SL |
| 13 | M | 11.7 | Left | good | N/A | upper | E | SL | N/A | N/A |
| 14 | F | 12.1 | Right | poor | 3 | full | SL | E | SL | E |
| 16 | M | 13.11 | Right | good | N/A | full | SL | E | SL | E |
| 17 | F | 15.7 | Left | good | N/A | full | SL | E | SL | E |
| 18 | M | 13.11 | Right | fair | N/A | 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 caries when orthodontic appliances were placed
[4]E = elastomeric-ligating bracket; SL = self-ligating bracket

***Higher Bacterial Plaque Load Was Observed Surrounding Elastomeric-Ligating Appliances:***

Table II illustrates the mean bacterial numbers for total bacteria and oral streptococci contained in plaque surrounding the SL and E brackets at both one week and five weeks post-bonding. In all cases described in Table 2, the bacterial numbers were greater in plaque surrounding the E bracket versus the SL bracket. It should be noted that significant statistical comparisons were identified primarily when using data pooled for both the maxillary and mandibular arch. Using the combined data set, higher total bacterial numbers were obtained surrounding the E brackets versus SL brackets at both one week ($p$=0.017) and five weeks ($p$=0.032) post-bonding. Total streptococci numbers were also higher in plaque surrounding the E brackets versus SL brackets at both one week ($p$=0.044) and five weeks ($p$=0.030) post-bonding. For all data groups examined, the standard deviations for the mean values contained in each group were quite high, most likely reflecting the variability in oral hygiene maintained among patients. Even though requests were made that no patients brush immediately prior to visits, we expect that not all patients were compliant. This is also consistent with the variable amounts of visible plaque collected between patients. Thus, in order to account for inter-patient variability, we used the split-mouth study design to also conduct comparisons of plaque bacteria contained within intra-arch bracket pairs in individual mouths.

**Table 2: Numbers of Total Bacteria and Streptococci on Teeth with Self-Ligating and Elastomeric Brackets**

| | One Week Post-Bonding | | | | | Five Weeks Post-Bonding | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Self-Ligating | | Elastomeric | | | Self-Ligating | | Elastomeric | | |
| Bacteria Type | Mean* | SD* | Mean* | SD* | $p$ | Mean* | SD* | Mean* | SD* | $p$ |
| Total Bacteria | 2.00 | 2.46 | 5.00 | 7.59 | 0.017** | 2.00 | 4.23 | 3.00 | 4.68 | 0.032** |
| Oral Streptococci | 0.70 | 1.17 | 2.00 | 4.02 | 0.044** | 0.50 | 1.37 | 2.00 | 4.05 | 0.030** |

* All listed mean and standard deviation values should be multiplied by $10^6$ to provide corrected values.
** Values that are statistically significant at the 95% confidence level ($p<0.05$).

Figure 9 illustrates intra-arch bracket pair comparisons divided on the basis of those exhibiting higher numbers of plaque bacteria on teeth bonded with E versus SL brackets. Histogram bar graphs are displayed as numbers of contrasts where bacterial numbers, for either total bacteria or oral streptococci, surrounding the E bracket was greater than bacterial numbers surrounding the SL bracket (E > SL), as well as the reverse comparison (SL > E).

Using these intra-arch bracket pair comparisons, in the majority of patients, at both the one week recall and the five week recall, teeth bonded with E brackets exhibited higher numbers of bacteria than teeth bonded with SL brackets. Specifically, greater numbers of patients (and arches derived from patients) exhibited higher levels of plaque bacteria surrounding the E appliances compared to the SL appliances (E>SL comparison) during the first recall visit ($p$=0.028; Figure 9). Interestingly, when the data was split into the maxillary and mandibular arch for the first recall visit, and examined to determine numbers of arches where plaque bacteria were higher on tooth surfaces surrounding the E appliance as compared to SL appliance (E>SL), differences were found to be nearly significant ($p$=0.052) for the maxillary arch but not for the mandibular arch ($p$=0.248). The difference observed for the maxillary arch may be potentially significant, but does not meet the theoretical threshold of significant differences ($p$<0.050). Also for the second recall visit, when using intra-arch bracket pair comparisons, there appears to be no statistically-significant differences in the numbers of arches where plaque bacteria was higher on tooth surfaces surrounding the E appliance as compared to SL appliance. In this case, the higher $p$ values may have been affected by the reduced statistical power of smaller numbers of patients who completed the study and participated in the second recall visit (n=14 patients) compared to the larger number of patients who initiated the study and were present for the first recall visit (n=18 patients).

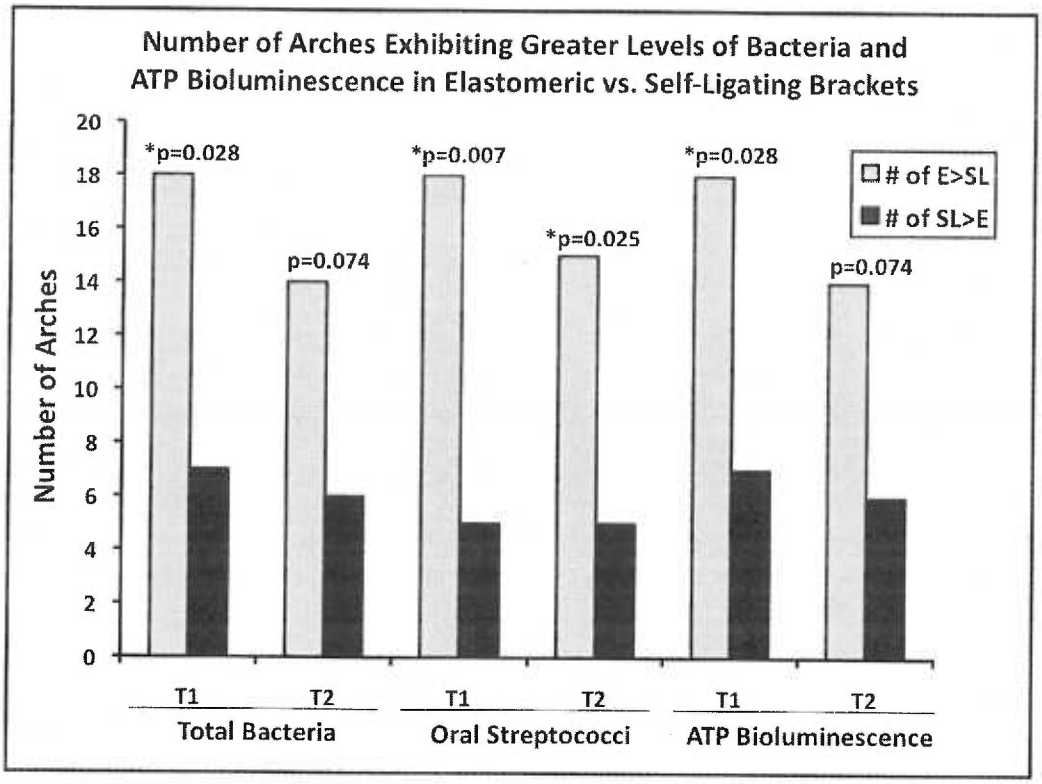



**Figure 9: Intra-arch comparison of bacterial numbers and ATP bioluminescence values obtained from plaque on tooth surfaces surrounding self-ligating versus elastomeric appliances.** Histograms depict numbers of arches, in intra-arch comparisons, where plaque bacteria (either total bacteria or oral streptococci) or ATP bioluminescence values are higher on tooth surfaces surrounding elastomeric compared to self-ligating appliances (E>SL; gray boxes) and the reverse comparison (SL>E; black boxes) for any given patient. Data is analyzed using numbers for the first and second recall visits (1 week and 5 weeks, post-bonding). *p* values are depicted for every comparison. Statistically-significant comparisons are denoted with an asterisk and are identified with *p* values $\leq$ 0.05.

In addition, when examining oral streptococci for the intra-arch comparisons, higher levels of streptococci were found in plaque surrounding the E bracket versus the SL bracket at both 1 week (p=0.007) and five weeks (p=0.025) post-bonding (Figure 9). These results are consistent with the comparison of the mean value determinations described in Table II. Thus, the use of elastomeric-ligation appliances promotes higher retention of plaque bacteria, including oral streptococci, at one week and potentially through five weeks post-bonding.

***Higher ATP-Driven Bioluminescence Levels Were Observed in Plaque Surrounding Elastomeric-Ligating Appliances:***

Figure 9 illustrates intra-arch bracket pair comparisons divided on the basis of those exhibiting higher levels of ATP-driven bioluminescence from plaque on teeth bonded with an E versus SL bracket. Histogram bar graphs are displayed as numbers of arches where ATP-driven bioluminescence obtained from plaque surrounding the E bracket was greater than corresponding RLU values from plaque surrounding the SL bracket (E > SL), as well as the reverse comparison (SL > E).

Like the data examining bacterial cell numbers, using intra-arch bracket pair comparisons, teeth bonded with E brackets, compared to SL brackets, generally exhibited higher ATP-driven bioluminescence values from plaque. Specifically, greater numbers of patients (and arches derived from patients) exhibited higher ATP-driven bioluminescence values from plaque surrounding the E appliances compared to SL appliances (E>SL comparison) during the first recall visit ($p$=0.028; Figure 9). Thus, as in the case of bacterial cell numbers, higher ATP-driven bioluminescence values were found in plaque surrounding the E appliances compared to the SL appliances.

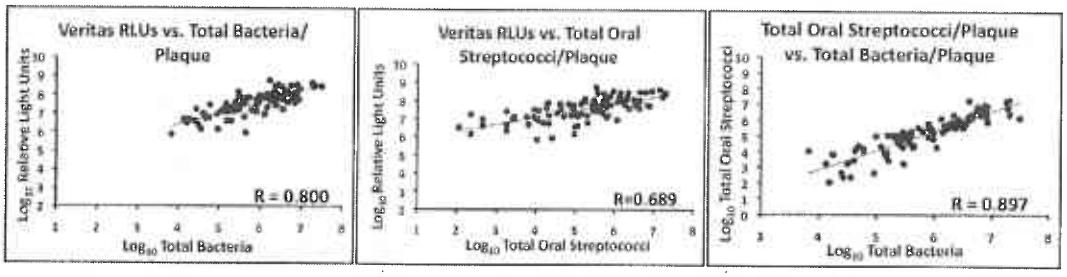
In the majority of patients (or more specifically in the majority of arches), individuals who were placed in the E>SL category for total bacteria were also found in the E>SL category for streptococci and for ATP-driven bioluminescence. There were some exceptions however, where individuals in the E>SL category for total bacteria were found in the SL>E category for either oral streptococci or ATP-driven bioluminescence. When these exceptions were excluded from the data set, we still found statistically-higher levels of bacterial load and ATP-driven bioluminescence observed in plaque surrounding the elastomeric-ligating appliances ($p<0.05$), consistent with the conclusions made when analyzing the complete data set.

***High Statistical Correlation Linking ATP-Driven Bioluminescence to Total Oral Bacteria and Total Oral Streptococci:***

Using plaque specimens collected from all patients, serial dilution plating of each specimen was conducted for quantification of total plaque bacteria using enriched medium (blood agar) and total streptococci using selective medium (mitis salivarius agar). When ATP-driven bioluminescence values were determined and compared to bacterial cell number, significant Pearson correlation coefficients of 0.800 and 0.689 were determined for total plaque bacteria and total plaque streptococci, respectively (with 1.0 being a perfect correlation; see Figure 10A, left and middle panels, respectively). When scatter plot analyses were conducted correlating total plaque bacteria with total plaque streptococci (Figure 10; right panel), increasing numbers of total plaque bacteria were found to track linearly with total plaque streptococci in a highly significant relationship ($r = 0.897$). When these ATP bioluminescence readings were analyzed using the combined set of plaque and saliva specimens, highly-significant correlation coefficients of 0.895 and 0.843 were identified for total oral bacteria and total oral streptococci,

respectively (Figure 10B, left and middle panels, respectively). When scatter plot analyses were conducted correlating total oral bacteria with total oral streptococci using the combined plaque and saliva data set (Figure 10B, right panel), increasing numbers of total oral bacteria were found to track almost linearly with total oral streptococci in a highly-significant relationship ($r = 0.940$).

**A. Scatter Plot Diagrams Using Plaque Specimens**




**B. Scatter Plot Diagrams Using Combined Plaque and Saliva Specimens**

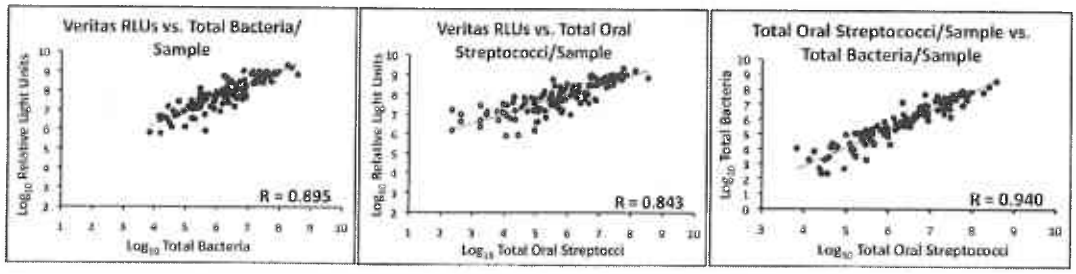



**Figure 10: Statistical Correlation Linking ATP-Driven Bioluminescence to Total Oral Bacteria and Total Oral Streptococci**

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

## DISCUSSION

Iatrogenic decalcification of tooth enamel and the development of visible white spot lesions are undesirable and unfortunate consequences of fixed orthodontic therapy, potentially undermining the esthetic benefits often achieved through the correction of a malocclusion. It is well documented that fixed appliances increase bacterial plaque accumulation and increase the risk potential for the development of white spot lesions (Gorelick,L. 1982; O'Reilly,M.M. 1987; Artun,J. 1986; Geiger,A.M. 1983; Mizrahi,E. 1982; Ogaard,B. 1989; Gwinnett,A.J. 1979; Forsberg,C.M. 1991; Rosenbloom,R.G. 1991; Huser,M.C. 1990). The development of the acid-etch bonding technique(Buonocore,M.G. 1955) and the subsequent orthodontic application via the bonding of brackets in lieu of full banded appliances, has not only facilitated the efficiency of orthodontic appliance construction, but also reduced the amount of tooth surface covered with appliances. Nonetheless, bonded orthodontic brackets hinder access for good oral hygiene and create microbial shelters, resulting in the accumulation of plaque. The appliance architecture, specifically the arch wire ligation method, serves as an additional factor influencing bacterial colonization. The results of the present study indicate that in the majority of patients, the tooth bonded with the self-ligating appliance exhibited lower numbers of bacteria than the tooth bonded with the elastomeric-ligating appliance.

Acid-producing bacteria colonize the tooth surface surrounding orthodontic appliances, leading to enamel demineralization and often causing an alteration in the appearance of the enamel surface (Gorelick,L. 1982; O'Reilly,M.M. 1987; Artun,J. 1986; Geiger,A.M. 1983; Mizrahi,E. 1982; Ogaard,B. 1989; Gwinnett,A.J. 1979) Gorelick et al. (1982). and Mizrahi et al. (1982) found maxillary incisors and first molars to be the teeth with the highest prevalence of

white spot lesions. In fact, Gorelick et al. (1982) found that the teeth with the highest individual incidence for formation of white spot lesions were maxillary lateral incisors, with the second most commonly affected tooth being the maxillary central incisors. Interestingly, the same researchers found the length of treatment to have little effect, with patients in treatment for 12-16 months experiencing the same incidence of white spot lesions as those involved in longer treatment schedules for up to 36 months.

Given these potential side effects, several approaches have been recommended to help prevent the accumulation of plaque bacteria and subsequent enamel damage around fixed appliances. Fluoride-releasing compounds (Ortendahl,T. 1997; Millett,D.T. 1996; Banks,P.A. 1997; Mitchell,L. 1992) and fluoridated elastomers (Mattick,C.R. 2001; Miura,K.K. 2007) have been introduced and demonstrate questionable success in providing sustained enamel protection. Home care regimens, such as daily sodium fluoride (NaF) mouth rinses, have demonstrated significant measures of protection, but suffer from potential non-compliance from individual users and requires additional cooperation from patients for maximum effectiveness (O'Reilly,M.M. 1987; Benson,P.E. 2005; Zachrisson,B.U. 1975).

In a scanning electron microscopic histological study, Sukontapatipark et al. (2001) found the area surrounding the bracket base was almost completely covered with a thick accumulation of bacteria within one week after the placement of appliances, and attributed this to excess composite, with adjacent smooth areas exhibiting a less mature monolayer of bacteria. In similar studies, Glatz, et al. (1985) and O' Reilly et al. (1987) demonstrated measurable histologic decalcification (up to 15% demineralization, to a depth of 50-75 μm) occurring around orthodontic appliances after only a month of placement. Furthermore, O'Reilly et al. (1987)

found that decalcification related to bonded orthodontic appliances occurred immediately around the appliance and not farther away along the facial surface.

Based on the observations that the maxillary lateral incisors demonstrate the highest prevalence of development of white spot lesions, ostensibly on the facial tooth surfaces at the immediate periphery of the brackets, we chose to use a circum-bracket plaque collection technique, a modification of the method utilized by Forsberg et al. (1991). We wanted to analyze the hygienic effects of the bracket ligation technique by studying and sampling plaque from tooth surfaces most affected and esthetically relevant, the anterior esthetic dentition.

The bonding of fixed orthodontic appliances hinders good oral hygiene, and creates new shelters for microbial colonization. During treatment, there is demonstrated increased retention in the amounts of *Streptococcus mutans* and lactobacilli in saliva and dental plaque (Rosenbloom,R.G. 1991; Forsberg,C.M. 1991). Several studies have evaluated the effect of fixed orthodontic appliances on microbial flora and periodontal status, but few studies have evaluated the manner of ligation as an additional factor(Forsberg,C.M. 1991; Sukontapatipark,W. 2001; Turkkahraman,H. 2005). Two studies that were conducted lacked randomization using a split-mouth design(Sukontapatipark,W. 2001; Turkkahraman,H. 2005)or lacked arch wire engagement because only one tooth was bonded in each quadrant(Sukontapatipark,W. 2001). This randomization is important because hygiene studies have noted differences in brushing habits relative to handedness(Rugg-Gunn,A.J. 1978; Thienpont,V. 2001). Specifically, right-handed people (and vice-versa for left-handed people) tend to brush better or spend more time brushing their contralateral sides. Furthermore, no published studies were conducted that compared differences in bacterial retention between traditional elastomeric-ligation versus self-ligation methods.

Forsberg et al. (1991) studied the effect of microbial plaque retention surrounding fixed appliances ligating with steel ligatures and elastomeric ties in 12 patients treated by fixed orthodontic appliances. Utilizing circum-bracket sampling techniques, Forsberg et al. (1991) found that the maxillary lateral incisors that were attached to archwires with elastomeric rings exhibited greater numbers of bacteria than incisors ligated with steel wires. They recommended that the use of elastomeric ligatures should be avoided in patients with poor oral hygiene because elastomeric ligation rings may significantly increase microbial accumulation on tooth surfaces adjacent to the brackets, leading to a predisposition for the development of dental caries and gingivitis. In contrast, Tukkahraman et al. (2005) found no significant differences in the numbers of microorganisms obtained from teeth ligated using similar techniques, either with elastomeric rings or with steel ligature wires. However, this study design was different from the design used by Forsberg et al. (1991) in that second premolars, not lateral incisors were sampled, and the allocation of brackets was not randomized. Thus, Tukkahraman et al. (2005) utilized less commonly-affected and less visible posterior teeth in the study design, making sampling difficult due to the short clinical crowns and gingival proximity. Furthermore, the method of plaque collection was qualitative in design, not the quantitative, circum-bracket technique used by Forsberg et al. (1991) and the present study. These contrasting study designs, as well as the different statistical analyses, may account for the differences in conclusions identified in these studies.

Based on the results of Forsberg et al. (1991) which indicated that there is reduced bacterial retention around brackets ligated with steel ligatures as opposed to elastomeric ties, it was a logical hypothesis that the complete absence of a ligature, that is, a self-ligating mechanism would presumably be equally as hygienic, if not better than a stainless steel ligature.

In the current study, the most common method of arch wire ligation, that of elastomeric ties, was chosen as the basis of comparison against the self-ligating mechanism. The placement of steel ligatures on all brackets is time consuming, and rarely done on a routine basis in the majority of current orthodontic practices. Further studies would have to be done to compare the difference between steel ligatures and self-ligating brackets.

It is important to note that the population under study included some patients that maintained exquisite oral hygiene, where minimal plaque was retained around the appliances, regardless of appliance type. While this behavior is desirable and encouraged for all our patients, it may not be representative of the level of oral hygiene maintained by patients outside of well-controlled clinical trials. Inclusion of patients exercising exceptionally good oral hygiene may potentially diminish true differences in plaque retention that may be observed between the appliances. It would be interesting to perform a similar study including patients who exercise inadequate oral hygiene, and are most affected and at risk for decalcification. Additionally, the nature of the study was described to patients at the time of the informed consent, with patients becoming aware of their inclusion in the study; thus, prior knowledge of their inclusion in this study may have had a confounding behavioral effect on the level of oral hygiene conducted by the patients.

The purposes of this longitudinal clinical study utilizing a "four quadrant," split-mouth design were to measure and compare the numbers of bacteria found in plaque surrounding two distinct orthodontic brackets, self-ligating versus elastomeric brackets. Based on the observations that the maxillary lateral incisors demonstrate the highest prevalence of development of white spot lesions, ostensibly on the facial tooth surfaces at the immediate periphery of the brackets, we chose to use a circum-bracket plaque collection technique, a

modification of the method utilized by Forsberg et al. (1991) Although the results indicate reduced retention of plaque bacteria surrounding the self-ligating appliances, it should be noted that any mechanics utilizing elastomeric chains or similar auxillaries in conjunction with the self-ligating appliances will presumably negate the beneficial effects of self-ligating appliances, possibly also diminishing other proposed purported benefits, such as reduced friction and lower force delivery. Thus, clinicians should be cognizant of this consideration when placing elastomerics over the self-ligating appliances in an effort to satisfy patient desires for colored 'bands' or in instances where elastomeric chains are to be in place for extended periods of time, as in space closing mechanics. Moreover, although the results of this 5-week study reflect reduced plaque retention around the self-ligating brackets, longer-term clinical trials should be conducted to ascertain a better understanding of the clinical significance of the use of different ligation methods.

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

## CONCLUSIONS

1. The results of this study indicate that self-ligating appliances promote reduced retention of oral bacteria, including streptococci, as compared to elastomeric-ligating appliances. In the majority of patients, the teeth bonded with a SL attachment exhibited fewer numbers of bacteria in plaque, as well as lower levels of ATP bioluminescence, than the teeth bonded with an E bracket.

2. ATP-driven bioluminescence values correlated significantly to numbers of oral bacteria and oral streptococci ($r$ = 0.895 and 0.843, respectively), indicating that ATP-driven bioluminescence may serve as a useful tool in the rapid, chair-side quantification of bacterial load and in the assessment of oral hygiene maintained during orthodontic treatment.

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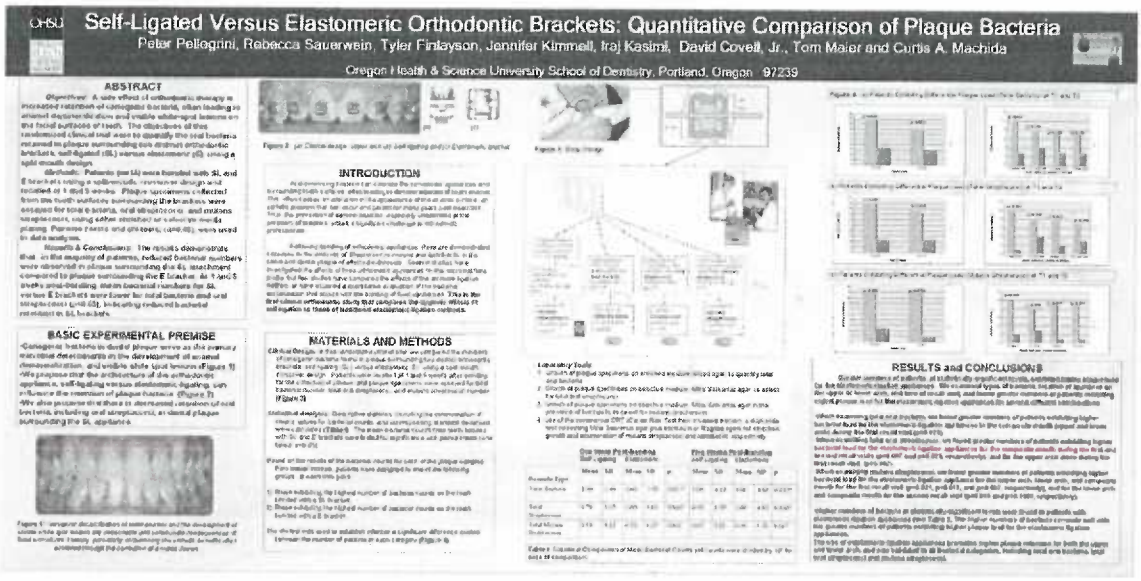
## APPENDICES:

## APPENDIX 1: AADR (American Association for Dental Research)

2008 **P Pellegrini**, R Sauerwein, T Finlayson, J Kimmell, I Kasimi, D Covell, Jr, T Maier, CA Machida. Self-Ligated vs. Elastomeric Orthodontic Brackets: Quantitative Comparison of Plaque Bacteria. American Association for Dental Research Annual Session, Poster Presentation. Dallas, Texas

## AADR Poster Presentation:



**AADR Abstract**:

Self-Ligated Versus Elastomeric Orthodontic Brackets: Quantitative Comparison of Plaque Bacteria

**Objectives:** A side effect of orthodontic appliances is increased retention of cariogenic bacteria, often leading to enamel demineralization and visible white-spot lesions on the facial surfaces of teeth. The objectives of this study were to compare the numbers and types of bacteria found in plaque surrounding two distinct orthodontic brackets, self-ligated versus elastomeric, using a split-mouth design.

**Methods:** Patients (males and females; ages 11-17) were bonded with a complementary mix of the self-ligated and elastomeric orthodontic brackets in opposite quadrants in upper (14 patients) and lower (12 patients) arches. Patients were recalled at 1 and 5 weeks after bonding for collection of plaque from standardized areas of the tooth surrounding the brackets. Plaque specimens were assayed for total bacterial number, total oral streptococci, and *Streptococcus mutans* number. Mean, standard error, and significance values (p; Student's *t*-test) were calculated.

**Results:** At one week post-bonding, the mean and standard error values for self-ligated versus elastomeric brackets were statistically lower for total bacteria (1.6 X 106 $\pm$ 4.79 X 105 versus 5.29 X 106 $\pm$ 1.46 X 106 bacteria, respectively; p=0.008) and for total oral streptococci (6.66 X 105 $\pm$ 2.23 X 105 versus 2.52 X 106 $\pm$ 9.16 X 105 streptococci, respectively; p=0.03). Similar statistical differences were identified at five weeks post-bonding between self-ligated and elastomeric brackets for both total bacteria and oral streptococci. Statistically-significant differences were also observed for total bacteria between the self-ligated and elastomeric

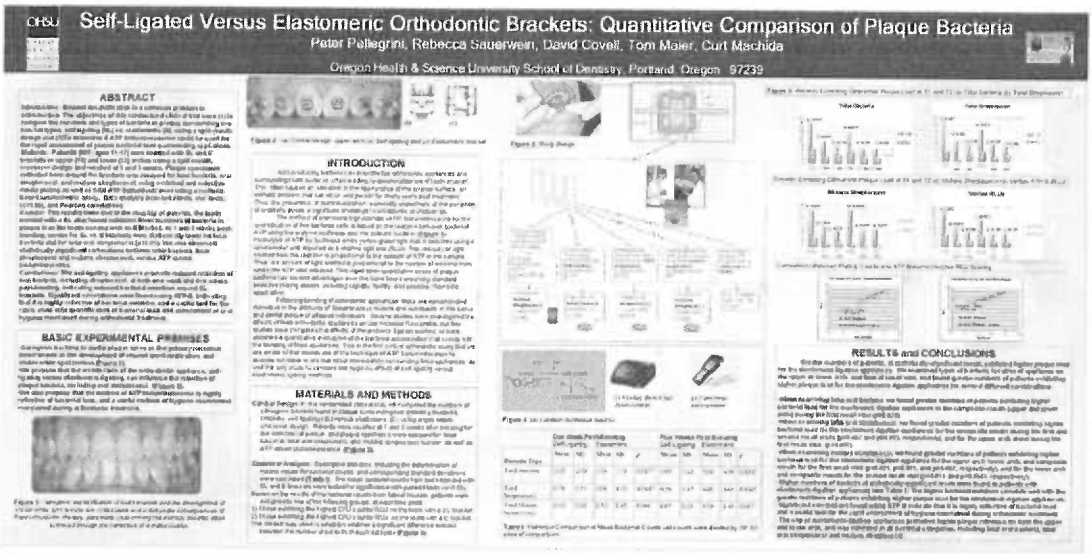
brackets in the upper arch ($p=0.01$) with no statistically-significant differences in the lower arch ($p=0.17$).

**Conclusions:** The self-ligated appliances promote reduced retention of oral bacteria, including streptococci, at both one week and five weeks post-bonding. Statistically-significant differences comparing plaque bacteria collected from self-ligated and elastomeric appliances appear to hold for the upper arch, but not for the lower arch.

## APPENDIX 2: AAO (American Association of Orthodontists)

2008 **P Pellegrini**. Self-Ligated Versus Elastomeric Orthodontic Brackets: Quantitative Comparison of Plaque Bacteria. American Association of Orthodontists Annual Session, Charley Schultz Resident Scholar Award Program, Poster Competition. Denver, Colorado

## AAO Poster Presentation:



**AAO Abstract:**

Self-Ligated Versus Elastomeric Brackets: Quantitative Comparison of Plaque Bacteria Using ATP-Bioluminescence

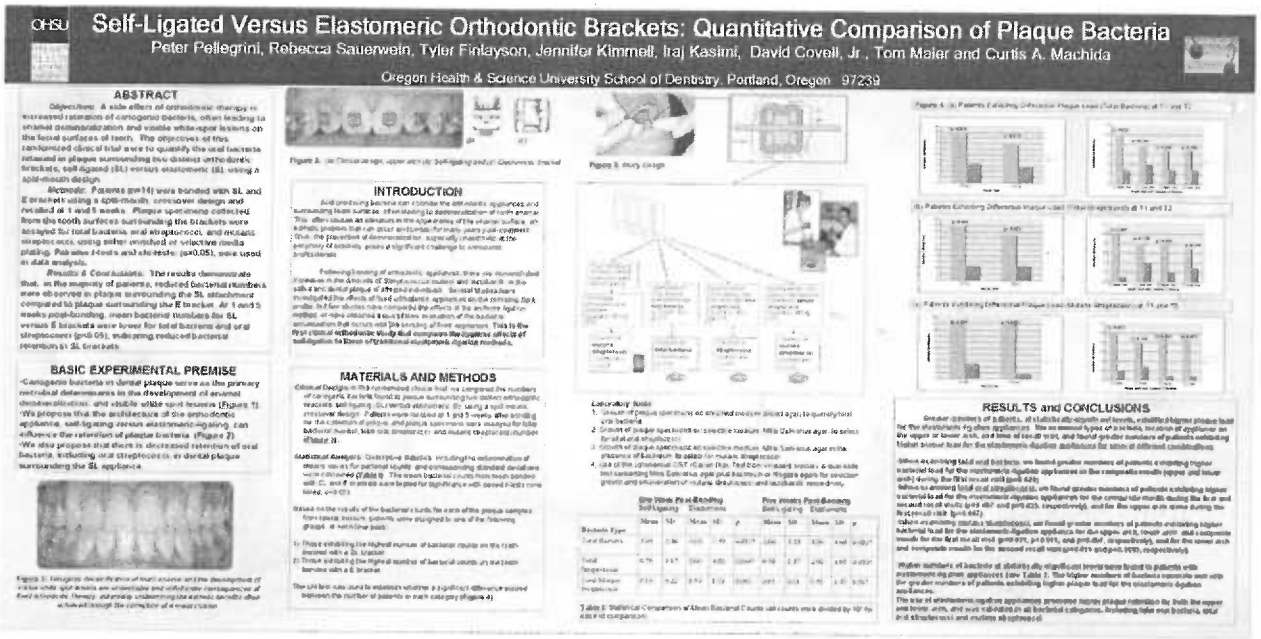
Objectives: Decalcification is a common problem in orthodontics. Aims of this randomized clinical trial were: 1) Compare numbers & types of plaque bacteria around self-ligated(SL) vs elastomeric(E) brackets 2) Analyze the use of ATP bioluminescence (ATP-B) for the assessment of bacterial load around appliances. Methods: Patients (n=14) were bonded with SL and E brackets using a split-mouth design and recalled at 1 and 5 weeks. Circum-bracket plaque was assayed for total bacteria, oral strep. and mutans strep. using selective media plating and luminometric assays for total ATP-B. Data analysis included t-tests, chi2-tests; ($\alpha$=0.05), and Pearson correlations. Results & Conclusions: At 1 and 5 weeks, means for SL vs E brackets were lower for total bacteria and oral strep. ($p<0.05$), indicating reduced bacterial retention in SL brackets. Significant correlations were found using ATP-B, indicating that it is highly reflective of bacteria counts and useful for orthodontic hygiene assessment.

## APPENDIX 3: IADR (International Association of Dental Research)

2008 R Sauerwein, **P Pellegrini**, T Finlayson, J Kimmell, I Kasimi, D Covell, Jr, T Maier, CA Machida. ATP Bioluminescence: Quantitative Assessment of Plaque Bacteria Surrounding Orthodontic Appliances. International Association of Dental Research Annual Session, Poster Presentation. Toronto, Canada

***Rebecca Sauerwein and Dr. David A. Covell, Jr. were presenters**

## IADR Poster Presentation:



**IADR Abstract**:

ATP Bioluminescence: Quantitative Assessment of Plaque Bacteria Surrounding Orthodontic Appliances

Rebecca Sauerwein[1*], Peter Pellegrini[2*+], Tyler Finlayson[1], Jennifer Kimmell[1,4], Iraj Kasimi[1], David Covell, Jr.[2], Tom Maier[1,3], and Curtis A. Machida[1]

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**Objectives:** The objectives of this study were to determine if ATP bioluminescence could be used for the rapid assessment and quantification of plaque bacterial load on tooth surfaces surrounding orthodontic appliances.

**Methods:** Patients (14 individuals; ages 11-17) were bonded with orthodontic brackets and then recalled at 1 and 5 weeks post-bonding for collection of plaque surrounding the orthodontic appliances. Plaque specimens were assayed for total bacterial number, total streptococci and mutans streptococci number, and total ATP bioluminescence using a luciferin-based luminometric assay. Pearson correlations were then calculated comparing bacterial cell numbers, as a composite population of all plaque specimens, versus ATP bioluminescence.

**Results:** We have identified strong to moderate statistical correlations between total plaque bacteria, total streptococci and mutans streptococci versus ATP-driven bioluminescence, and have calculated significant r values of 0.808, 0.674, and 0.651, respectively. High correlation coefficients were also determined when measuring ATP bioluminescence from plaque specimens using a hand-held luminometer that can be used at chair-side. Additionally, the majority of bacteria in the plaque specimens were composed of streptococci; this determination was

supported with a strong correlation coefficient of 0.895.

**Conclusions:** We conclude that ATP-driven bioluminescence is highly predictive of the numbers of total plaque bacteria and total streptococci, and by statistical extension, is also reflective of the numbers of cariogenic mutans streptococci. This study supports consideration of ATP bioluminescence as a useful tool for the rapid, chair-side quantification of plaque bacteria load and as a general assessment indicator of oral hygiene maintained during orthodontic treatment.

## APPENDIX 4: *AJO-DO* (American Journal of Orthodontics and Dentofacial Orthopedics)

- **Intial Manuscript Submitted to AJO-DO July 1, 2008**
- **Final Manuscript (appended here) accepted for publication August 20, 2008**
- **Final Manuscript accepted for publication August 20, 2008**
  - **(NOTE 1: Figures have been embedded in the following document and may not reflect manuscript placement)**
  - **(NOTE 2: This document includes changes also made and submitted in the proofing stage done through September, 2008)**

# Plaque retention by self-ligating versus elastomeric orthodontic brackets:

# Quantitative comparison of oral bacteria and detection using ATP-driven bioluminescence


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## ABSTRACT

**Introduction:** Enamel decalcification is a common problem in orthodontics. The objectives of this randomized clinical study were to enumerate and compare plaque bacteria surrounding two bracket types, self-ligating (SL) versus elastomeric-ligating (E), and to determine if ATP-driven bioluminescence could be used in the rapid assessment of bacterial load in plaque.

**Methods:** Patients (ages 11-17) were bonded with SL and E brackets in maxillary (14) and mandibular (12) arches using a split-mouth design and had recall visits at 1 and 5 weeks post-bonding. Plaque specimens were assayed for oral bacteria, and subjected to ATP-driven bioluminescence determinations using a luciferin-based assay.

**Results:** In the majority of patients, teeth bonded with SL attachments exhibited fewer numbers of bacteria in plaque than teeth bonded with E brackets. At 1 and 5 weeks post-bonding, means for SL versus E brackets were statistically lower for total bacteria and for oral streptococci ($p<0.05$). ATP bioluminescence values were statistically correlated to numbers of total oral bacteria and oral streptococci, and had correlation coefficients of 0.895 and 0.843, respectively.

**Conclusions:** The self-ligating appliances promote reduced retention of oral bacteria, and ATP bioluminescence may serve as a useful tool in the rapid quantification of bacterial load and in the assessment of oral hygiene maintained during orthodontic treatment.

## INTRODUCTION

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

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

Following bonding of orthodontic appliances, there are documented increases in the amounts of the cariogenic microorganisms, *Streptococcus mutans* and lactobacilli, in saliva and dental plaque of treated individuals.[10-13] While several studies have investigated the effects of fixed orthodontic appliances on the microbial flora profile, few studies have compared the effects of the bracket architecture, specifically the arch wire ligation method,[10,14,15] or have obtained a quantitative evaluation of the bacterial accumulation that occurs with the bonding of fixed appliances.

Rapid ATP-driven (adenosine triphosphate) bioluminescence assays have long been used as a quantitative measure of microbial numbers, and more recently in dental plaque.[16-19] Bioluminescence assays

measuring energy metabolites, including ATP, have been shown to have high correlations with plaque mass obtained from both humans and animal subjects.[16-20]

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

## MATERIALS AND METHODS

***Test Subjects*:** Fourteen patients (12 with full appliances, 2 with appliances on the maxillary arch only) successfully completed the study out of the originally-enrolled eighteen (18) patients, with four patients being excluded because of failure to adhere to the selection criteria or failure to keep one or more appointments for specimen collection. The criteria for inclusion in this study were age, with participants being at dental age 12 or older ($x$ = 13.9 years, range 11.7- 17.2 years) and demonstrated ability to maintain adequate oral health. All patients selected were diagnosed as requiring fixed appliance orthodontic therapy and scheduled for treatment in the Orthodontics Clinic at the Oregon Health & Sciences University (OHSU). Patients who were pregnant, diabetic, using any mouth rinses or interacting medications, including antibiotic therapy within the three months prior to the study, were excluded from participation. Participants were assigned study identifier numbers that were accessible only to the study investigators and were kept in a notebook locked in the Department of Orthodontics.

***IRB and Human Subjects Consent:*** This study was reviewed and approved by the OHSU Institutional Review Board (IRB). In addition to the consent form for routine orthodontic care currently in use in the OHSU Orthodontic Clinic, the parent/guardian of each subject selected for participation was given a second consent form specifically relating to the clinical study. We requested that participants also refrain from eating or drinking one hour prior to the sampling appointments. The study called for no additional treatment or procedures not normally performed in the routine oral care provided during initial bonding or orthodontic adjustment visits.

***Description of Randomized Split-Mouth Design and Subject Preparation*:** The doctor assigned for the plaque collection (PP) utilized a standardized collection technique, 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 hand piece. Patients were given oral

hygiene instruction and fluoridated toothpaste and toothbrush, and asked to refrain from other oral hygiene supplements for the duration of the study. At the initial visit, one half of each arch, either left or right sides, was randomly assigned to receive the experimental bracket with the opposite side serving as the control (Figure 1A). Specifically, for each arch, the left or right lateral incisors randomly received either the experimental "self-ligating bracket" (0.022"; In-Ovation-R®, GAC International, Bohemia, NY [Figure 1B]) or the control "elastomeric-ligating bracket" (0.022"; Mini-Ovation®, GAC [Figure 1C]); the latter brackets were ligated with silver-colored elastomeric ligatures (AlastiKs®, 3M Unitek, Monrovia, CA). The appliances were directly bonded using composite resin, with all but the lateral incisors bonded with self-ligating brackets (0.022"; In-Ovation-R®).

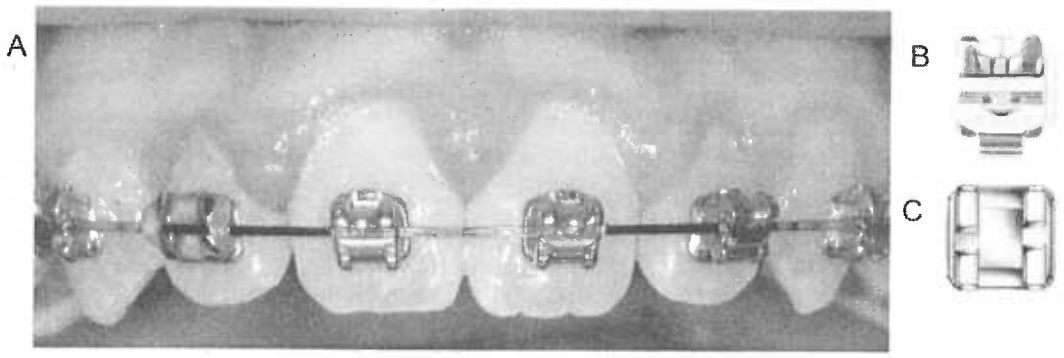


**Figure 1: Experimental design and description of self-ligating and elastomeric appliances. A.** Experimental design on an enrolled patient maxillary arch; **B.** Self-ligating bracket, Innovation-R,® (GAC International, Bohemia, NY); **C.** Traditional elastomeric-ligating bracket, Mini-Ovation,® (GAC).

***Procedures for Collection of Plaque:*** The sampling investigators utilized a standardized protocol to collect specimens: At each designated sampling visit, the operator carefully removed/disengaged the elastomeric tie/ligation mechanism, and removed the archwires. Plaque specimens were collected from the labial surfaces immediately surrounding the orthodontic brackets of the maxillary and mandibular lateral incisors utilizing a sterilized dental scaler with the same tip dimensions (# 8/9 Orban DE hoe

scaler, Hu-Friedy, Chicago, Illinois). Given the area of increased decalcification generally occurring immediately adjacent to the brackets, a four-pass technique was utilized to move the instrument tip around the circumference of the bracket at the bracket-tooth interface (Figure 2). Four passes, one along the tooth at the bracket interface at the gingival, mesial, distal and occlusal, were used to avoid overloading the instrument tip. All of the specimens from each tooth (left and right incisor for both maxillary and mandibular arch) were placed into four individual tubes that had anonymous coding, and sealed for transport to the laboratory. The coding of specimens assured blinding of laboratory personnel and helped minimize experimental bias.

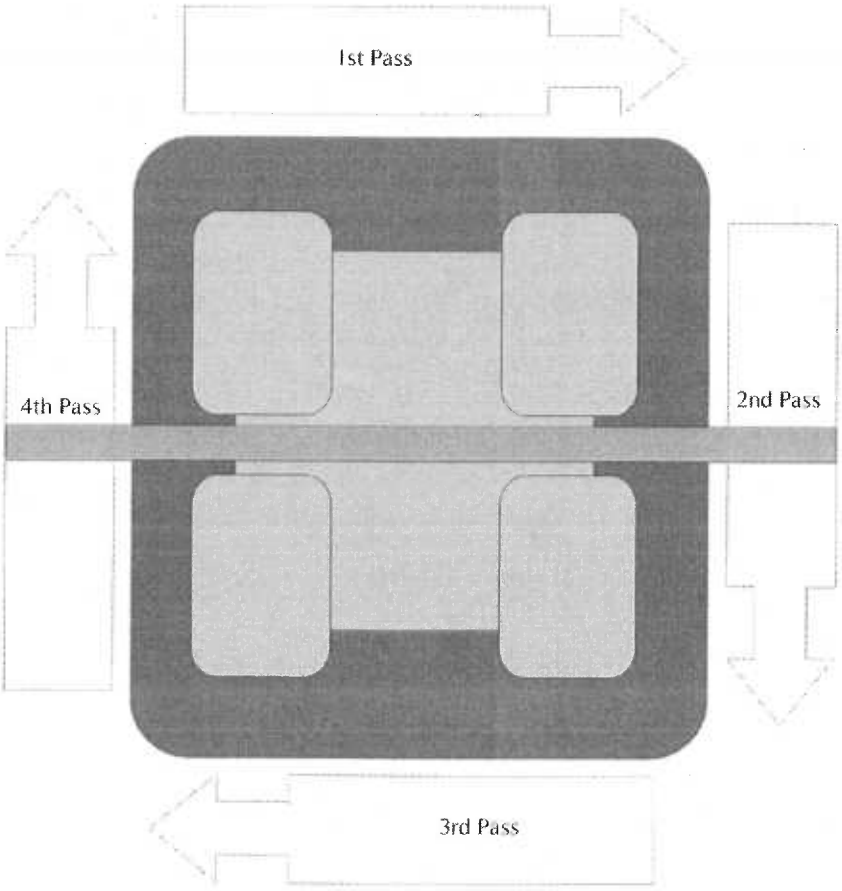


**Figure 2: Four-pass sampling technique.** A standardized, sterilized instrument tip is moved circumferentially around the bracket. Wire is pictured, and the ligation mechanism is carefully removed prior to sampling.

***Saliva Collection Procedures:*** Participants were given a chewing gum-shaped paraffin wax tablet, and instructed to chew the tablet for 1-5 minutes or until several ml of saliva was collected and expelled into a sterile calibrated collection container. The sample was coded with the unique participant identifier, and transferred to the laboratory for evaluation. At the $T_0$ bonding appointment, collection of saliva occurred prior to placement of appliances.

***Microbiological Analyses of Specimens:*** Five specimens (4 plaque plus stimulated saliva specimens) were collected per subject at each appointment after bonding ($T_1$ and $T_2$: 1 week and 5 weeks after bonding, respectively), with the exception of the bonding appointment ($T_0$) where only saliva specimens were collected. Each plaque specimen was diluted in 1 ml of phosphate buffered saline (PBS), in the presence of glass beads, and dispersed by vigorous agitation on a rocker platform (37°C, 10 minutes). Dispersed plaque samples were then subjected to 10-fold serial dilutions in PBS and then plated on enriched blood agar (PML Microbiologicals, Wilsonville, OR) to determine total bacterial numbers. Total oral streptococci numbers were determined by limiting dilution plating on mitis salivarius agar (MSA; Difco™; Becton, Dickinson and Company, Sparks, MD), which utilizes high sucrose and vital dyes as selective agents. MSA will select for numerous strains of oral streptococci with varying abilities to generate acid and effects on decalcification potential. All platings were conducted in quadruplicate, and plates exhibiting colony numbers between 50 and 500 were counted and averaged to determine mean values.

***ATP-Driven Bioluminescence of Specimens:*** Using the luciferin substrate and luciferase enzyme, bacterial ATP can be quantitated by measuring the release of visible light.[16] ATP contained in bacteria from plaque specimens was determined with the use of the BacTiter Glo Microbial Cell Viability Assay kit (Promega, Madison, WI; product number G8231), with ATP-driven bioluminescence measured by the Veritas Microplate luminometer (Turner Biosystems; San Diego, CA). 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 or absorbance at 600 nM wavelength measured with Novaspec II Visible spectrophotometer). The Veritas luminometer has a 5-fold dynamic range in RLU readouts.

***Statistical Analysis:*** Eighteen patients were enrolled in an effort to account for dropouts (Table 1). Fourteen patients completed the study, with twelve in full appliances, and 2 receiving maxillary appliances only. Descriptive statistics, including the determination of means values for bacterial counts, and corresponding standard deviations were calculated. The mean bacterial counts and ATP-driven bioluminescence determinations (in RLUs) from teeth bonded with SL and E brackets were tested for significant differences using paired *t*-tests (one-tailed, with p values $< 0.05$ considered statistically significant). Based on the results of the bacterial counts and ATP-driven bioluminescence determinations for each of the plaque samples from lateral incisors, contrasting bracket pairs within each arch were assigned to one of the following groups: 1) Those exhibiting the highest number of bacterial counts or RLUs on the tooth bonded with a SL bracket (SL> E) and 2) Those exhibiting the highest number of bacterial counts or RLUs on the tooth bonded with an E bracket (E>SL). The $chi^2$-test and distribution were used to establish whether a significant difference existed between the numbers of contrasts in each category.[10]

## RESULTS

***Patient Demographics and Positions of Appliances Used in the Study:*** Table I describes the patient population in this study, including the randomized allocation of brackets bonded to the lateral incisors. Note that seven patients had E brackets placed on the maxillary right quadrant and SL brackets placed on the maxillary left quadrant, and that seven patients had SL brackets placed on the maxillary left quadrant and E brackets placed on the maxillary right quadrant. Also, in all cases for any given patient, the appliance type was switched in its left-right orientation when appliances were placed in the mandibular arch. Ten individuals were right handed, three individuals were left handed, and one was ambidextrous. Plaque was collected from lateral incisors in the maxillary right quadrant (tooth #7), maxillary left quadrant (tooth #10), mandibular left quadrant (tooth #23), and mandibular right quadrant (tooth #26) (also defined as upper right (UR), upper left (UL), lower left (LL) and lower right (LR) quadrants, respectively, in Table 1).

**Table 1: Patient Demographics and Placement of Brackets on Selected Teeth**

| Patient #[1] | Gender | Age | Handedness | Oral Health | Average Time (hrs) brushing before visits[2] | Orthodontic Treatment[3] | (UR) Tooth 7 Bracket Type[4] | (UL) Tooth 10 Bracket Type[4] | (LL) Tooth 23 Bracket Type[4] | (LR) Tooth 26 Bracket Type[4] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 14.6 | Right | good | 1 | full | E | SL | E | SL |
| 2 | M | 14.11 | Right | good | 1 | full | E | SL | E | SL |
| 3 | F | 16.1 | Right | good | 1 | full | SL | E | SL | E |
| 4 | F | 14 | Right | good | 3 | full | SL | E | SL | E |
| 5 | F | 17.2 | Ambidextrous | good | 1 | upper | E | SL | N/A | N/A |
| 6 | F | 12.2 | Right | good | 1 | full | SL | E | SL | E |
| 7 | F | 12.9 | Right | good | 1 | full | E | SL | E | SL |
| 8 | F | 15.9 | Right | good | 2 | full | E | SL | E | SL |
| 12 | F | 12.11 | Left | good | N/A | full | E | SL | E | SL |
| 13 | M | 11.7 | Left | good | N/A | upper | E | SL | N/A | N/A |
| 14 | F | 12.1 | Right | poor | 3 | full | SL | E | SL | E |
| 16 | M | 13.11 | Right | good | N/A | full | SL | E | SL | E |
| 17 | F | 15.7 | Left | good | N/A | full | SL | E | SL | E |
| 18 | M | 13.11 | Right | fair | N/A | 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 caries when orthodontic appliances were placed
[4]E = elastomeric-ligating bracket; SL = self-ligating bracket

***Higher Bacterial Plaque Load Was Observed Surrounding Elastomeric-Ligating Appliances:*** Table 2 illustrates the mean bacterial numbers for total bacteria and oral streptococci contained in plaque surrounding the SL and E brackets at both one week and five weeks post-bonding. In all cases described in Table 2, the bacterial numbers were greater in plaque surrounding the E bracket versus the SL bracket. It should be noted that significant statistical comparisons were identified primarily when using data

pooled for both the maxillary and mandibular arch. Using the combined data set, higher total bacterial numbers were obtained surrounding the E brackets versus SL brackets at both one week ($p$=0.017) and five weeks ($p$=0.032) post-bonding. Total streptococci numbers were also higher in plaque surrounding the E brackets versus SL brackets at both one week ($p$=0.044) and five weeks ($p$=0.030) post-bonding. For all data groups examined, the standard deviations for the mean values contained in each group were quite high, most likely reflecting the variability in oral hygiene maintained among patients. Even though requests were made that no patients brush immediately prior to visits, we expect that not all patients were compliant. This is also consistent with the variable amounts of visible plaque collected between patients. Thus, in order to account for inter-patient variability, we used the split-mouth study design to also conduct comparisons of plaque bacteria contained within intra-arch bracket pairs in individual mouths.

**Table 2: Numbers of Total Bacteria and Oral Streptococci on Teeth With Self-Ligating and Elastomeric Brackets**

| | One Week Post-Bonding | | | | | Five Weeks Post-Bonding | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Self-Ligating | | Elastomeric | | | Self-Ligating | | Elastomeric | | |
| Bacteria Type | Mean* | SD* | Mean* | SD* | *p* | Mean* | SD* | Mean* | SD* | *p* |
| Total Bacteria | 2.00 | 2.46 | 5.00 | 7.59 | 0.017** | 2.00 | 4.23 | 3.00 | 4.68 | 0.032** |
| Oral Streptococci | 0.70 | 1.17 | 2.00 | 4.02 | 0.044** | 0.50 | 1.37 | 2.00 | 4.05 | 0.030** |

* All listed mean and standard deviation values should be multiplied by $10^6$ to provide corrected values.
** Values that are statistically significant at the 95% confidence level ($p<0.05$).

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

Using these intra-arch bracket pair comparisons, in the majority of patients, at both the one week recall and the five week recall, teeth bonded with E brackets exhibited higher numbers of bacteria than teeth bonded with SL brackets. Specifically, greater numbers of patients (and arches derived from patients) exhibited higher levels of plaque bacteria surrounding the E appliances compared to the SL appliances (E>SL comparison) during the first recall visit ($p$=0.028; Figure 3). Interestingly, when the data was split into the maxillary and mandibular arch for the first recall visit, and examined to determine numbers of arches where plaque bacteria were higher on tooth surfaces surrounding the E appliance as compared to SL appliance (E>SL), differences were found to be nearly significant ($p$=0.052) for the maxillary arch but not for the mandibular arch ($p$=0.248). The difference observed for the maxillary arch may be potentially significant, but does not meet the theoretical threshold of significant differences ($p$<0.050). Also for the second recall visit, when using intra-arch bracket pair comparisons, there appears to be no statistically-significant differences in the numbers of arches where plaque bacteria was higher on tooth surfaces surrounding the E appliance as compared to SL appliance. In this case, the higher $p$ values may have been affected by the reduced statistical power of smaller numbers of patients who completed the study and participated in the second recall visit (n=14 patients) compared to the larger number of patients who initiated the study and were present for the first recall visit (n=18 patients).

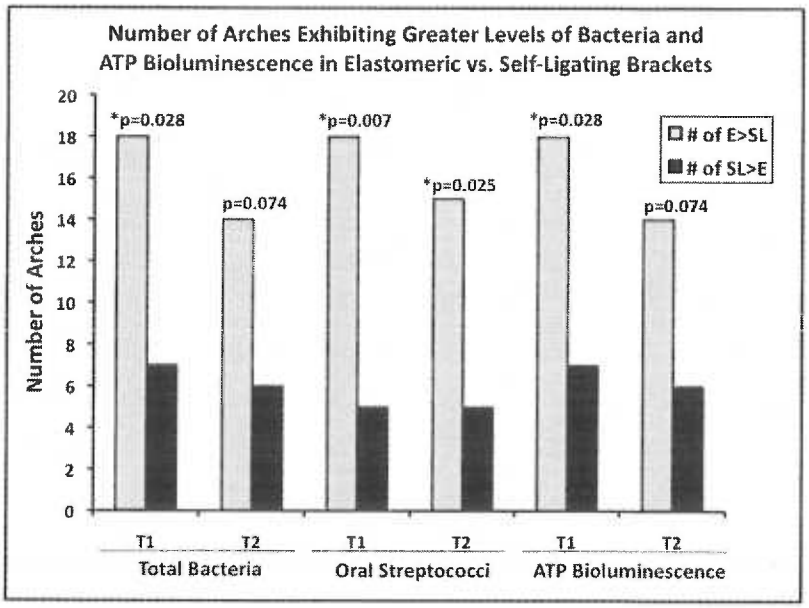


**Figure 3: Intra-arch comparison of bacterial numbers and ATP bioluminescence values obtained from plaque on tooth surfaces surrounding self-ligating versus elastomeric appliances.** Histograms depict numbers of arches, in intra-arch comparisons, where plaque bacteria (either total bacteria or oral streptococci) or ATP bioluminescence values are higher on tooth surfaces surrounding elastomeric compared to self-ligating appliances (E>SL; gray boxes) and the reverse comparison (SL>E; black boxes) for any given patient. Data is analyzed using numbers for the first and second recall visits (1 week and 5 weeks, post-bonding). *p* values are depicted for every comparison. Statistically-significant comparisons are denoted with an asterisk and are identified with *p* values $\leq$ 0.05.

In addition, when examining oral streptococci for the intra-arch comparisons, higher levels of streptococci were found in plaque surrounding the E bracket versus the SL bracket at both 1 week (p=0.007) and five weeks (p=0.025) post-bonding (Figure 3). These results are consistent with the comparison of the mean value determinations described in Table 2. Thus, the use of elastomeric-ligation appliances promotes higher retention of plaque bacteria, including oral streptococci, at one week and potentially through five weeks post-bonding.

***Higher ATP-Driven Bioluminescence Levels Were Observed in Plaque Surrounding Elastomeric-Ligating Appliances:*** Figure 3 illustrates intra-arch bracket pair comparisons divided on the basis of those exhibiting higher levels of ATP-driven bioluminescence from plaque on teeth bonded with an E versus SL bracket. Histogram bar graphs are displayed as numbers of arches where ATP-driven bioluminescence obtained from plaque surrounding the E bracket was greater than corresponding RLU values from plaque surrounding the SL bracket (E > SL), as well as the reverse comparison (SL > E).

Like the data examining bacterial cell numbers, using intra-arch bracket pair comparisons, teeth bonded with E brackets, compared to SL brackets, generally exhibited higher ATP-driven bioluminescence values from plaque. Specifically, greater numbers of patients (and arches derived from patients) exhibited higher ATP-driven bioluminescence values from plaque surrounding the E appliances compared to SL appliances (E>SL comparison) during the first recall visit ($p$=0.028; Figure 3). Thus, as in the case of bacterial cell numbers, higher ATP-driven bioluminescence values were found in plaque surrounding the E appliances compared to the SL appliances.

In the majority of patients (or more specifically in the majority of arches), individuals who were placed in the E>SL category for total bacteria were also found in the E>SL category for streptococci and for ATP-driven bioluminescence. There were some exceptions however, where individuals in the E>SL category for total bacteria were found in the SL>E category for either oral streptococci or ATP-driven bioluminescence. When these exceptions were excluded from the data set, we still found statistically-higher levels of bacterial load and ATP-driven bioluminescence observed in plaque surrounding the elastomeric-ligating appliances ($p<0.05$), consistent with the conclusions made when analyzing the complete data set.

***High Statistical Correlation Linking ATP-Driven Bioluminescence to Total Oral Bacteria and Total Oral Streptococci:*** Using plaque specimens collected from all patients, serial dilution plating of each

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

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

## DISCUSSION

Iatrogenic decalcification of tooth enamel and the development of visible white spot lesions are undesirable and unfortunate consequences of fixed orthodontic therapy, potentially undermining the esthetic benefits often achieved through the correction of a malocclusion. It is well documented that fixed appliances increase bacterial plaque accumulation and increase the risk potential for the development of white spot lesions.[1-7,10,11,21] The development of the acid-etch bonding technique[22] and the subsequent orthodontic application via the bonding of brackets in lieu of full banded appliances, has not only facilitated the efficiency of orthodontic appliance construction, but also reduced the amount of tooth surface covered with appliances. Nonetheless, bonded orthodontic brackets hinder access for good oral hygiene and create microbial shelters, resulting in the accumulation of plaque. The appliance architecture, specifically the arch wire ligation method, serves as an additional factor influencing bacterial colonization. The results of the present study indicate that in the majority of patients, the tooth bonded with the self-ligating appliance exhibited lower numbers of bacteria than the tooth bonded with the elastomeric-ligating appliance.

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

Given these potential side effects, several approaches have been recommended to help prevent the accumulation of plaque bacteria and subsequent enamel damage around fixed appliances. Fluoride-

releasing compounds[23-26] and fluoridated elastomers[29,30] have been introduced and demonstrate questionable success in providing sustained enamel protection. Home care regimens, such as daily sodium fluoride (NaF) mouth rinses, have demonstrated significant measures of protection, but suffer from potential non-compliance from individual users and requires additional cooperation from patients for maximum effectiveness.[2,27,28]

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

Based on the observations that the maxillary lateral incisors demonstrate the highest prevalence of development of white spot lesions, ostensibly on the facial tooth surfaces at the immediate periphery of the brackets, we chose to use a circum-bracket plaque collection technique, a modification of the method utilized by Forsberg et al.[10] We wanted to analyze the hygienic effects of the bracket ligation technique by studying and sampling plaque from tooth surfaces most affected and esthetically relevant, the anterior esthetic dentition.

The bonding of fixed orthodontic appliances hinders good oral hygiene, and creates new shelters for microbial colonization. During treatment, there is demonstrated increased retention in the amounts of *Streptococcus mutans* and lactobacilli in saliva and dental plaque.[10,11] Several studies have evaluated the effect of fixed orthodontic appliances on microbial flora and periodontal status, but few studies have

evaluated the manner of ligation as an additional factor.[10,14,15] Two studies that were conducted lacked randomization using a split-mouth design[14,15] or lacked arch wire engagement because only one tooth was bonded in each quadrant.[15] This randomization is important because hygiene studies have noted differences in brushing habits relative to handedness.[32,33] Specifically, right-handed people (and vice-versa for left-handed people) tend to brush better or spend more time brushing their contralateral sides. Furthermore, no published studies were conducted that compared differences in bacterial retention between traditional elastomeric-ligation versus self-ligation methods.

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

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

It is important to note that the population under study included some patients that maintained exquisite oral hygiene, where minimal plaque was retained around the appliances, regardless of appliance type. While this behavior is desirable and encouraged for all our patients, it may not be representative of the level of oral hygiene maintained by patients outside of well-controlled clinical trials. Inclusion of patients exercising exceptionally good oral hygiene may potentially diminish true differences in plaque retention that may be observed between the appliances. It would be interesting to perform a similar study including patients who exercise inadequate oral hygiene, and are most affected and at risk for decalcification. Additionally, the nature of the study was described to patients at the time of the informed consent, with patients becoming aware of their inclusion in the study; thus, prior knowledge of their inclusion in this study may have had a confounding behavioral effect on the level of oral hygiene conducted by the patients.

The purposes of this longitudinal clinical study utilizing a "four quadrant," split-mouth design were to measure and compare the numbers of bacteria found in plaque surrounding two distinct orthodontic brackets, self-ligating versus elastomeric brackets. Based on the observations that the maxillary lateral incisors demonstrate the highest prevalence of development of white spot lesions, ostensibly on the facial tooth surfaces at the immediate periphery of the brackets, we chose to use a circum-bracket plaque

collection technique, a modification of the method utilized by Forsberg et al.[10] Although the results indicate reduced retention of plaque bacteria surrounding the self-ligating appliances, it should be noted that any mechanics utilizing elastomeric chains or similar auxillaries in conjunction with the self-ligating appliances will presumably negate the beneficial effects of self-ligating appliances, possibly also diminishing other proposed purported benefits, such as reduced friction and lower force delivery. Thus, clinicians should be cognizant of this consideration when placing elastomerics over the self-ligating appliances in an effort to satisfy patient desires for colored 'bands' or in instances where elastomeric chains are to be in place for extended periods of time, as in space closing mechanics. Moreover, although the results of this 5-week study reflect reduced plaque retention around the self-ligating brackets, longer-term clinical trials should be conducted to ascertain a better understanding of the clinical significance of the use of different ligation methods.

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

## CONCLUSIONS

1. The results of this study indicate that self-ligating appliances promote reduced retention of oral bacteria, including streptococci, as compared to elastomeric-ligating appliances. In the majority of patients, the teeth bonded with a SL attachment exhibited fewer numbers of bacteria in plaque, as well as lower levels of ATP bioluminescence, than the teeth bonded with an E bracket.

2. ATP-driven bioluminescence values correlated significantly to numbers of oral bacteria and oral streptococci ($r$ = 0.895 and 0.843, respectively), indicating that ATP-driven bioluminescence may serve as a useful tool in the rapid, chair-side quantification of bacterial load and in the assessment of oral hygiene maintained during orthodontic treatment.

**ACKNOWLEDGEMENTS**

This work was supported by the OHSU Foundation Orthodontic Fund, Oral Biotech, and the Oregon Clinical and Translational Research Institute (OCTRI). RS is the 2008 OCMID Student Research Scholar in Caries Microbiology. JK was a 2007 OCTRI Summer Research Fellow. DC, TM, and CM are supported by the OHSU School of Dentistry. The authors thank and acknowledge Dr. Tyson Buck for providing orthodontic clinical support, Iraj Kasimi and Janna Hickerson for preparation of plating medium and laboratory support, and RS for overseeing the overall laboratory analysis and for the development of the figures and graphs used in this manuscript. The authors thank Tom Shearer, Jack Clinton, Alexis Pellegrini and Kim Kutsch for their encouragement in the development of this project.

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