# ATP Bioluminescence: A Clinical Tool to Measure Plaque Retention on Tooth Surface around Orthodontic Brackets

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**Abstract**:
**Objective:** The purpose of the present study was to quantify dental plaque retention on tooth surface around different types of orthodontic brackets using **ATP** bioluminescence measurement technique**. Methods**: the sample consisted of 30 subjects selected from the out-patient clinic at Orthodontic Department, Faculty of Dentistry, Suez Canal University, Ismailia, Egypt. These selected patients required fixed appliance orthodontic therapy. The subjects were divided into three groups each bonded with different bracket type; stainless steel **G1**, Ceramic (G2), and self-ligating (G3); each ten subjects. A split mouth design was assigned as half of each arch, either the left or the right side, was randomly assigned to receive the experimental bracket, with the opposite side as the control. For each arch, either the left or the right premolars was selected to receive the experimental measurements. The measurements were in relative light units (RLU) values. **Results**: All groups showed non-significant measurements but G2 (ceramic brackets) recorded the highest measurement 104001.90 ± 17423.85 and the lowest measurement (36774.40 ± 8636.22) was observed in **G3** (self-ligating bracket) after 4 weeks. **Conclusion**: The Self-ligating brackets are more hygienic. ATP-driven bioluminescence technique could serve as a useful tool in the rapid chair-side quantification of bacterial load and in the assessment and monitoring of oral hygiene during orthodontic treatment.

**[**Muktar A. Elalem, Nabeel E. Shamaa, Mohamed Adel Nadim. **ATP Bioluminescence: A Clinical Tool to Measure Plaque Retention on Tooth Surface around Orthodontic Brackets.** *J Am Sci* 2013;9(7):335-339]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 41

**Keywords:** Self ligating brackets; Ceramic brackets; Stainless steel brackets (st st); ATP bioluminescence; Relative Light Units (RLU); Dental plaque.

## 1. 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 [1.2]. Bonded brackets have many advantages because of better esthetics, ease of placement and removal.[3-5]. Nonetheless, bonded orthodontic brackets impede good oral hygiene, resulting in plaque accumulation and significantly increased risks for enamel decalcification.

Several studies have investigated the effects of fixed orthodontic appliances on the microbial flora profile, few studies have compared the effects of bracket architecture specifically, the archwire ligation method or have obtained a quantitative evaluation of the bacterial accumulation that occurs with the bonding of fixed appliances [6-9].

Rapid adenosine triphosphate (ATP)-driven bioluminescence assays have long been used as a quantitative measure of microbial numbers and more recently in dental plaque. Bioluminescence assays measuring energy metabolites, including ATP, have been shown to have high correlations with plaque mass obtained from both human and animal subjects[10, 11].Bacterial metabolism requires ATP and this can be used as a measure of viable bacteria in biological samples. ATP bioluminescence is a sensitive technique, which detects bacteria by measuring light emitted when their ATP reacts with firefly luciferase and luciferin. Standard microbiological techniques for culturing bacteria from samples take a minimum of 5 days to complete. ATP bioluminescence assay reduces this testing time to 24 hours[12].

In this clinical study, we quantify dental plaque retention on tooth surface around different orthodontic brackets by demonstrating the use of ATP-driven bioluminescence as an innovative tool for (immediate) rapid chair-side enumeration.

## 2.Subjects and Method

The sample consisted of 30 subjects selected from the outpatient clinic at Orthodontic Department, Faculty of Dentistry, Suez Canal University for whom required fixed appliance orthodontic therapy. The patients were divided into three equal groups **A**(stainless steel brackets, Gemini RothBrackets, 3M

Unitek Monrovia, Calif.),**B** (Ceramic brackets Inspire ICE™, Ormco Brackets) and **C**(self-ligating brackets).A split mouth design was assigned as half of each arch, either left or right side was randomly assigned to receive the experimental bracket, with the opposite side as control. For each arch, left or right premolars were selected to receive the experimental measurements (Fig.1)**.**

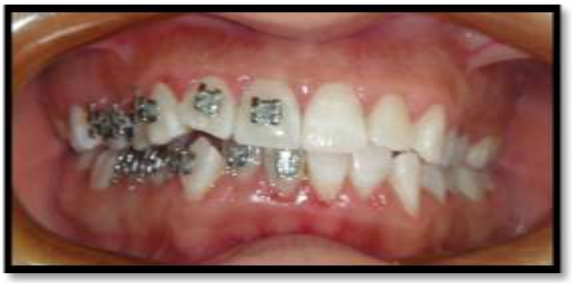

**Figure 1: Split mouth design (right side as measure, left side as control)**

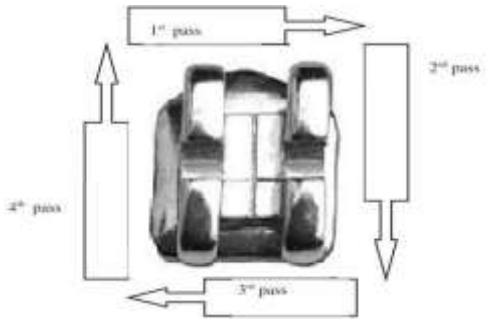


**Figure 2: Four-pass sampling technique: a standardized and sterilized swab tip is moved circumferentially around the** bracket.

The patients were given oral hygiene instructions, fluoridated toothpaste and a toothbrush, and asked not to use other oral hygiene supplements during the study, also requested the patients to refrain from eating or drinking 1 hour before the sampling appointments. All teeth were polished with coarse-grade prophylaxis paste (*AHL Generic Prophylaxis Paste Advanced Healthcare Ltd Tonbridge, Kent, TN11 8JU, UK),*with a rubber cup and a slow-speed hand piece. Acid etching was performed with 35% orthophosphoric acid (Transbond™ XT 35% Phosphoric Acid, 3M Unitek Monrovia, Calif CA 91016) for 20 seconds, and following the application of adhesive primer (Transbond™ XT Light Cure Adhesive Primer, 3M Unitek Monrovia, Calif CA 91016), and the bracket were directly bonded by using light cure composite resin (Transbond™ XT Light Cure Adhesive Paste, 3M Unitek Monrovia, Calif CA 91016) .

**Sample collection:**

A standardized collection technique was used for the sample collection. Two specimens were collected per subject at each appointment, the initial bracket bonding appointment (T0), after 1 week (T1) and after 4 weeks (T2). Plaque specimens were collected from the labial surfaces immediately surrounding the orthodontic brackets of premolars teeth with 3M™ Clean-Trace™ ATP surface test. All specimens from each tooth were placed into individual tubes with anonymous coding and naming to minimize experimental bias. A four-pass technique the area immediately adjacent to the brackets was used to move swab tip around the circumference of the bracket at the gingival, mesial, distal, and occlusal aspects (Fig. 2). The tooth surface around the selected brackets was swabbed and no touch to the gingiva to avoid false reading.

**Sample processing:**

The swab was embedded immediately into the swab kit which contains luciferin-luciferase enzyme to be analyzed for ATP bioluminescence.Reading of the results in RLU are gained directly as the luminometer is displayed.

This luminometer is dependent on adenosine triphosphate (ATP) as a source of energy which is found in all organic matter that can be easily recorded and measured. Calibration of RLU and correlation against optical density absorb at proper wavelength with visible spectrophotometer (luminometer). The idea depends on release of phosphate group which in turn cause release of energy [(13)]. Organic residues are rich in adenosine triphosphate ATP and provide micro-organisms with nutrients to grow. When ATP is brought into contact with the firefly reagent combination, luciferin-luciferase, a reaction takes place which results in production of light [(14)].The data or readings can be regarded as measurement of the light intensity. All test samples were counted 2 times in each visit.

**Statistical analysis:**

All data were processed with SPSS®; version 20.0.0 for Windows. Descriptive statistics, including means, standard deviations and t-test for with statistical significance level 95% level of confidence ($P < 0.05$).

## 3. Results

The total comparison of ATP bioluminescence values from plaque on tooth surfaces surrounding ***st st***, ***ceramic*** and ***SL*** brackets recorded the same level of measurement upon immediate measurement after bonding. A high ATP values were observed in the three groups by increasing the time as noticed after

one week. The ceramic bracket ***G2*** showed the ***highest*** measurement after 4 weeks (104001.90 ± 17423.85) relative to the other two groups ***G1***&***G3.*** Meanwhile, the ***lowest*** measurement was observed in **G3*SL*** after 4 weeks (36774.40 ± 8636.22). **Group 1** ***st st*** brackets recorded an intermediate behavior between the other two types of brackets (Table 1&Fig. 3).

Analysis using *one sample t-test* for RLU values from the surrounding of the three bracket types (3 groups) showed no statistical significance in bioluminescence ($p \leq 0.05$) among the three groups regarding the three phases of the study (Table 2).No obvious pattern favoring one bracket type over the other.

**Table1: Total ATP bioluminescence values on tooth surfaces surrounding the brackets of the three groups.**

| | N | G 1 | | G 2 | | G 3 | |
|---|---|---|---|---|---|---|---|
| | | Mean | ±SD | Mean | ±SD | Mean | ±SD |
| Immediate | 10 | 4529.50 | 1593.77 | 3803.00 | 2434.35 | 4810.00 | 2824.87 |
| 1 week | 10 | 29731.80 | 17990.07 | 20783.80 | 10460.17 | 12791.00 | 7423.47 |
| 4 weeks | 10 | 60834.70 | 28470.04 | 104001.90 | 17423.85 | 36774.40 | 8636.22 |

**G 1**: Stainless steel brackets, **G 2:** Ceramic brackets, **G 3**: Self-ligating brackets

**Table 2: t-test analysis for immediate, one week and 4 weeks RLU values from the surrounding of the three bracket types.**

| | Test Value = 5 | | | | | |
|---|---|---|---|---|---|---|
| | t | df | Sig. (2-tailed) | Mean Difference | 5% Confidence Interval of the Difference | |
| | | | | | Lower | Upper |
| ***Immediate*** | | | | | | |
| G1 | 8.977 | 9 | .000 | 4524.50 | 4492.00 | 4556.10 |
| G2 | 4.934 | 9 | .001 | 3798.00 | 3748.37 | 3847.64 |
| G3 | 5.379 | 9 | .000 | 4805.00 | 4747.40 | 4862.60 |
| ***One week*** | | | | | | |
| G1 | 5.225 | 9 | .001 | 29726.80 | 29359.99 | 30093.61 |
| G2 | 6.282 | 9 | .000 | 20778.80 | 20565.52 | 20992.08 |
| G3 | 5.447 | 9 | .000 | 12786.00 | 12634.64 | 12937.36 |
| ***4 weeks*** | | | | | | |
| G1 | 6.757 | 9 | .000 | 60829.70 | 60249.21 | 61410.19 |
| G2 | 18.875 | 9 | .000 | 103996.90 | 103641.64 | 104352.16 |
| G3 | 13.464 | 9 | .000 | 36769.40 | 36593.31 | 36945.49 |

**G 1**: Stainless steel brackets, **G 2:** Ceramic brackets, **G 3**: Self-ligating brackets *n:10 Significance level $p \leq 0.05$*

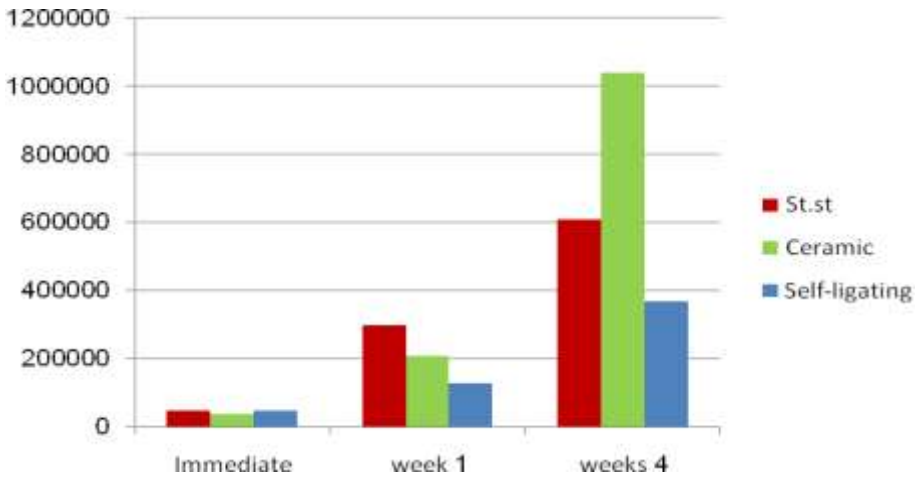


**Figure 3: Total ATP bioluminescence values on tooth surfaces surrounding the brackets of the three groups.**
**G 1**: Stainless steel brackets, **G 2:** Ceramic brackets, **G 3**: Self-ligating brackets

**4.Discussion**

This current *in-vivo* study was designed to quantify dental plaque retention on tooth surface around different types of orthodontic brackets using ATP bioluminescence measurement technique by using direct technique. Hence, bioluminescence technique was chosen for assessing bacterial colonization in this investigation as it represents a rapid and convenient mean on screening microbial samples.

Bacterial metabolism requires ATP and this can be used as a measure of viable bacteria in biological samples. ATP bioluminescence is a sensitive technique, which detects bacteria by measuring light emitted when their ATP reacts with firefly luciferase and luciferin.

In comparison of all previous microbiological techniques for culturing bacteria from samples the take a minimum of 5 days to complete. ATP bioluminescence assay reduces this testing time to 24 hours. This method could indicate good correlations among biofilm image areas and animmediate count of colony forming units.

**Relationship between plaque retention around different designs of orthodontic brackets and ATP-driven bioluminescence techninique:**

Many studies have evaluated the effect of fixed orthodontic appliance on microbial flora and periodontal status **Corbett *et al.,*** [3]**; Rosenbloom and Tinanoff** [4]**;**.**Sukontapatipark *et al.,*** [6]**.**However, only a few studies evaluated the effect of bracket type and design as an additional factor. Nonetheless, bonded orthodontic brackets hinde raccess for good oral hygiene and create microbial shelters, resulting in the accumulation of plaque.

No obvious pattern favoring one bracket type over the others (three groups). The results of this study showed no significance difference ($P \leq 0.05$) table 2**.** This study showed higher mean of ATP values for group B; ceramic brackets, while lower values were measured with **G3**; self-ligating brackets (Table 2). The findings (Fig.3) show that there was a trend to higher plaque accumulation around ceramic brackets **G2**. This result gives support to the recommendation that the use of ceramic brackets should be avoided in patients with inadequate oral hygiene because this type of bracket may be more prone to induce dental caries and gingivitis.

The results of the present study are in accordance with another related study by **Buck *et al.,*** [13] in which they described increased plaque retention with elastomeric-ligated brackets at 5 weeks post-bonding, there were no significant differences in bacterial numbers or ATP-driven bioluminescence values surrounding the elastomeric-ligated **vs** self-ligating brackets. Also, it is in agreement with **Pellegrini *et al.,*** [12]regarding theSL brackets that indicate fewer bacteria than those of stainless steel brackets.

In another research by **Forsberg *et al.,*** [5]registered the bacterial count on two occasions before insertion of the fixed appliance, and 6 weeks after the period of active treatment. Their results showed that, in the majority of patients, the incisor which was attached to the arch-wire with an elastomeric ring, exhibited a greater number of micro-organisms in the plaque than the incisor ligated with steel wire. But in the current study using ATP-driven bioluminescence evaluation had been recorded and investigated immediately after bonding, one week and after 4 weeks (active). This ways helps to investigate and monitor the biofilm behavior precisely.

Finally, the ATP-driven bioluminescence technique could be considered more convenient, reliable to measure and quantify the bacterial colonization resulted from plaque retention around orthodontic brackets.

**Conclusions**

1. No obvious statistical significant difference of plaque retention favoring any of the tested brackets; self-ligating, stainless steel and ceramic; was found.
2. Self-ligating brackets showed lowest ATP mean values than stainless steel and ceramic brackets, while ceramic brackets showed higher ATP values than other types in all tested time intervals (one and 4 weeks).
3. Under standardized conditions ATP-driven bioluminescence technique might serve as a useful tool in the rapid chair-side quantification of bacterial load, monitoring and assessment of the oral hygiene during orthodontic treatment

**Acknowledgment**

The authors would like to express special thanks to 3M Egypt company office, for kindly giving all technical support and supplying the 3M™ Clean-Trace™ Luminometer; for free measurements; used inthe present study.

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6/4/2013