Research Article

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A Comparison of Streptococcus Mutants Biofilm Retention on two Different Types of Brackets by two Different Ligation Method: An In-Vitro Study

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ABSTRACT

Background: The aim of the study was to evaluate *Streptococcus mutans* (ATCC 25157 strain) biofilm retention on the orthodontic metallic and ceramic brackets with 2 different ligation techniques using colony-counting method.

Methods and Materials: The extracted premolars for orthodontic purpose were taken and were stored in normal saline solution. The 80 extracted premolars were divided into 4 groups: Group MM– Conventional PEA metal brackets with .022 in slot with elastomeric module (n=20), Group ML– Conventional PEA metal brackets with .022 in slot with steel wire ligatures (n=20), Group CM– Conventional PEA ceramic brackets with .022 in slot with elastomeric module (n=20), Group CL– Conventional PEA ceramic brackets with .022 in slot with steel wire ligatures (n=20). The tooth samples were acid etched and brackets were bonded with light cured adhesive. The tooth samples were then immersed in Glucose Broth containing *S.mutans* and incubated aerobically for 72 hours at 37°C to allow bacterial accumulation. The bacteria were then counted using the digital colony counter.

Results: The digital colony counter showed significant statistical difference ($p < 0.05$) in bacterial adhesion between the four groups. Brackets ligated with elastomeric modules had more bacterial adhesion when compared to steel wire ligature group. **Conclusion** - Fixed orthodontic appliances significantly increase the retention of biofilm regardless the type of bracket system chosen. The result of the present study indicates that metallic brackets with steel ligature had least amount of biofilm retention when compared to elastomeric module and ceramic brackets.

Keywords: Ligation techniques, Bacterial adhesion, esthetic brackets, S. Mutans.

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INTRODUCTION

Bacterial adhesion to biomaterials and the ability of many microorganisms to form biofilm on foreign bodies are well-known steps in the pathogenesis of oral infections. Orthodontic appliances also serve as a medium for plaque accumulation, thereby increasing the level of microorganisms in the oral cavity. The oral environment with fixed orthodontic appliances provides proper conditions for the colonization of oral microorganisms; patients have difficulty in maintaining adequate oral hygiene, and the appliances provide additional places for bacterial binding. Of the many orthodontic appliances, brackets could play a significant role in enamel demineralization because they are attached to the dentition throughout the entire period of orthodontic treatment, and their complex design provides a unique environment that impedes proper access to the tooth surfaces for cleaning[1-4].

Patient undergoing orthodontic treatment are at higher risk for enamel demineralization due to plaque accumulation adjacent to the appliances. In addition, estimates of white spot lesions in the orthodontically treated population range from 12.6% to 50% of patients[5-6].

Cariogenic mutant streptococci are currently classified into six species *Streptococci cricetus, Streptococci mutans, Streptococci sobrinus, Streptococci downei, Streptococccci ferns, Streptococci macaque*. Among these *Streptococcus mutans* and *streptococcus sobrinus* have been associated with smooth surface caries. Patient who undergo orthodontic therapy have increased retentive sites for *Streptococcus mutans* strain and increased retention of food particles, which may lead to increase proportions and absolute numbers of salivary mutans which is an important component of the biofilm formation on human teeth that are associated with plaque formation, dental caries and white spots on tooth surface which may hamper the patient satisfaction during and post orthodontic therapy[7].

Prevention of these lesions is an important concern for orthodontists. Furthermore, the bracket material could play a role in the degree of bacterial adhesion and plaque accumulation as well as in the risk of caries development. The initial affinity of bacteria to solid surfaces is due mostly to electrostatic and hydrophobic interactions. Surfaces with high surface free energy more easily attract bacteria such as *S mutans*[8].In a study by Eliades et al[9], stainless steel presented the highest critical surface tension and can be expected to have a higher plaque retaining capacity. Metallic orthodontic brackets have been found to induce specific changes in the oral environment such as reduced levels of pH, increased plaque accumulation, and elevated *S mutans* colonization.

Information about the adhesion of cariogenic streptococci to orthodontic brackets can help to determine a means for preventing enamel demineralization. Although the physical and mechanical properties of orthodontic wires have been extensively studied, few studies have evaluated bacterial adhesion based on their surface characteristics. Also there is little in the orthodontic literature about the bacterial adhesion to bracket based on their method of ligation.

Many studies[10-22] have evaluated the effect of fixed orthodontic appliances on microbial flora and periodontal status however, only a few studies[23-27] have evaluated the type of ligation as an additional factor. The wires are secured/ ligated to the bracket by three methods; (a) ligation with steel wires, (b) ligation with elastomeric ligatures and (c) self-ligating brackets. Many studies documented; suggest that the elastomeric ligatures increase the retention of dental biofilm compared with the two other methods. Thus the present study was designed with an aim to evaluate bacterial adhesion on different types of brackets and different method of ligation.

MATERIALS AND METHOD

The 80 extracted premolars for orthodontic purpose were taken and were stored in normal saline solution and were divided into 4 groups:

Group – A – Conventional PEA metal brackets with .022 in slot with elastomeric or rubber module
(n=20)

Group –B – Conventional PEA metal brackets with .022 in slot with steel wire ligatures (n=20)

Group – C – Conventional PEA ceramic brackets with .022 in slot with elastomeric or rubber module
(n=20)

Group –D – Conventional PEA ceramic brackets with .022 in slot with steel wire ligatures (n=20)

Adhesion of streptococcus mutans

The teeth were then acid etched for 40 sec, rinsed with water and the air-dried. Primer was applied and cured for 20 sec. Brackets were then bonded using light cured adhesive.Each tooth sample was placed into a tube containing 3 ml of the prepared bacterial suspensions of S. *mutans* (ATCC 25157) and incubated aerobically for 72 hrs at 37^0C. After washing three times with phosphate-buffered saline (PBS), each specimen was transferred to a new tube containing 3 ml PBS. The adhered bacteria were then detached by scraping method with a cotton swab. The suspension was then serially diluted up to 10^{-1} to 10^{-3} and 100 µl of each dilution was spread on blood agar plate. After incubation for 48hrs, bacterial colony on each plate was counted. Colony counts were expressed as a colony-forming unit (CFU) per unit area (cm^2) of the growth plate. The values obtained were tabulated and then send for statistical evaluation.

Statistical Analysis

The data was subjected to statistical analysis using SPSS (Statistical Package for Social Sciences) version 20.0 statistical analysis software. The descriptive statistics including the mean, standard deviation, minimum and maximum values were calculated for each of the four experimental groups. One way ANOVA was used to compare the mean colony forming bacterial units per unit volume by the four groups. Tukey HSD-test was used to compare the individual mean between all four groups.

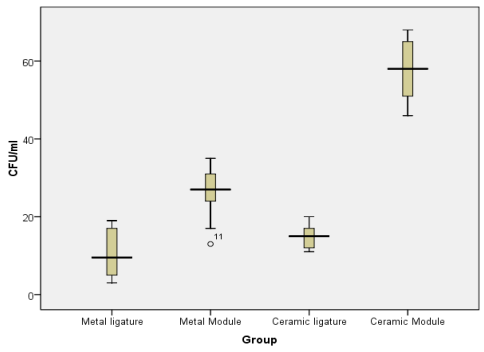
RESULTS

The aim of the study was to evaluate retention on two different types of brackets by two different ligation method. There was no significant difference in the measurements of bacterial colony formation between the metal ligature and ceramic ligature group. However a significant difference was seen between all the remaining groups.

Table 1: Multiple Comparisons by Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	P value	95% Confidence Interval	
					Lower Bound	Upper Bound
Metal ligature vs	Metal Module	-15.600	2.807	.000*	-23.16	-8.04
Metal ligature vs	Ceramic ligature	-4.500	2.807	.390***	-12.06	3.06
Metal ligature vs	Ceramic Module	-47.100	2.807	.000*	-54.66	-39.54
Metal Module vs	Ceramic ligature	11.100	2.807	.002**	3.54	18.66
Metal Module vs	Ceramic Module	-31.500	2.807	.000*	-39.06	-23.94
Ceramic ligature vs	Ceramic Module	-42.600	2.807	.000*	-50.16	-35.04

*p < 0.001 highly significant,**p<0.05 Significant,***p>0.05 Not Significant



DISCUSSION

Enamel demineralization adjacent to orthodontic brackets has been a great concern to orthodontists, because white spot lesions lead to esthetic problems. Previous studies showed that orthodontic brackets harbor significant amounts of plaque, and plaque accumulates around the brackets even in patients with good oral hygiene. However, relatively little work has been done on the possible role of orthodontic brackets in the adhesion of cariogenic streptococci and enamel demineralization. Ahn et al[14] found that different strains of cariogenic streptococci strains have different amounts of adhesion, even though they belong to the same species. Eliades et al[11] measured the surface free energy of the bracket raw materials and found that ceramic and plastic raw materials had lower surface tension than the stainless steel raw materials. The higher adhesion to the titanium brackets might be due to their rougher surfaces than the other brackets. The surface texture of titanium brackets was reported to be much rougher than that of the stainless steel brackets. This study was performed to determine amount of bacterial adhesion to different bracket and ligation technique. Forsberg et al[23] first demonstrated the method of ligation of 2 different types of ligation on fixed appliances with steel wires and elastomeric rings with its effect to microbial colonization around them. They found that lateral incisors that were attached to archwire with E-rings had greater number of microorganisms to that attached to steel wires. They also recommended the use of steel wires in patients with bad to poor oral hygiene as E-rings can increase the bacterial counts on tooth surface and cause dental caries and gingivitis. Sukontapatipark et al[28] employed 2 methods of ligation with E-rings and steel ligature and used SEM to evaluate the bacterial colonization with fixed metal appliances in premolar teeth and reported that both elastomeric rings and steel ligature had bacterial colonies adhesion and moreover reported that the method of ligation did not seem to influence on microorganisms on either composite resin or enamel surfaces. On the other hand Turkkahraman et al[13] presented an in-vivo study that revealed, elastomeric ligature had higher number of bacterial organisms to steel ligatures but the differences between them were not statistically significant and could be ignored. With regard to present study, it aimed at evaluating three different ligating techniques instead of two and also used premolar teeth for the study. Gracez et al[20] presented a study evaluating the biofilm adhesion to orthodontic brackets by 3 methods of ligation and used OCT(optical coherence Tomography) images to confirm them along with microbiologic sampling. The results obtained were similar to the present study. In the present study the biofilm retention to different orthodontic brackets by two different methods of ligation were assessed and it was found that elastomeric module had higher retention of biofilm when compared to self-ligating brackets and steel ligatures. Self-ligating brackets have greater biofilm retention due to presence of clips, hinges and other retention areas in the bracket design when compared to steel wire ligatures which has least biofilm retention. The biofilm retention is found to be greatest in elastomeric module because they are polyurethanes, thermosetting polymer products which are not inert materials and are affected by heat, moisture and contact with salivary and oral enzymes. Water acts as a plastisizer of these materials by weakening the intermolecular forces, leading to chemical degradation. Elastomeric modules which are stretched and placed onto the brackets have a tendency to imbibe moisture and enlarge to which the bacteria are attracted.

CONCLUSION

The following conclusions can be drawn from the present study:

1. Fixed orthodontic appliances significantly increase the retention of biofilm regardless the type of bracket system chosen.
2. The result of the present study indicates that steel ligature had least amount of biofilm retention when compared to elastomeric module.
3. Ceramic brackets are more prone for bacterial adhesion.
4. In both types of brackets more bacterial adhesion was seen in elastomeric ligature group.
5. Non significant difference in bacterial adhesion was seen between ligature group.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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