

Evaluation of Micro-organism in Ligated Metal and Self-ligating Brackets using Scanning Electron Microscopy: An *In Vivo* Study

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Abstract:

Background: The objective of the study was to determine the sites of plaque accumulation and to compare the plaque accumulated with metal and self-ligating orthodontic brackets in order to know which bracket type had a higher plaque retaining capacity.

Materials and Methods: The study was done on 20 subjects who were scheduled for orthodontic treatment including extraction of four premolars and fixed orthodontic appliances. Mesh-backed edgewise metal brackets ligated with steel ligatures and self-ligating brackets were bonded to the premolars to be extracted using composite (Transbond XT, 3M). The subjects were told to continue their normal oral hygiene regimen. Teeth were extracted at 1, 2, and 3 weeks after bracket bonding. Plaque attached to the buccal surfaces was stained using plaque disclosing agent. The teeth were then immersed in fixative containing 4% formaldehyde and 1% glutaraldehyde in phosphate buffer for 24 h, followed by 0.1 M phosphate buffer for 12 h. The specimens were then mounted on aluminum stubs, and sputter coated with gold prior to Scanning electron microscopy examination.

Results: The results showed that increased retention of plaque in metal brackets ligated with steel ligatures and comparatively less in self-ligating brackets at the base of the brackets.

Conclusions: This study highlights that higher retention of plaque in metal brackets ligated with steel ligatures and comparatively less plaque retention in self-ligating brackets. Excess composite around the bracket base is the critical site of plaque accumulation associated with fixed appliances due to its rough surface texture.

Key Words: Micro-organisms, orthodontic brackets, scanning electron microscopy

Introduction

The use of various active and passive components of the fixed appliances have been breeding grounds for bacterial colonization, hereby challenging the need for proper oral hygiene maintenance.

Adhesion of microorganisms to surfaces is a result of electrostatic interactions and Van-der-Walls forces.¹ Although it is clear that initial attachment is an important factor governing further colonization, the mechanism of attachment and those of subsequent adhesion may differ significantly.² Once attachment is established, additional factors may dictate further colonization. Decreased wettability may inhibit direct adhesion and colonization of bacteria on to the appliances.³

Patients who undergo orthodontic therapy have oral ecologic changes such as a low pH environment, increased retentive sites and increased retention of food particles, which may lead to increased number of salivary *Streptococcus mutans*.⁴ Understanding of the *S. mutans* levels in patients before, during, and after orthodontic therapy may help to determine caries risk levels and may thus lead to appropriate preventive or antimicrobial therapy.^{1,5,6}

Design and surface characteristics of both orthodontic attachments and composite may influence plaque retention. Composites used as direct bonding adhesive have a polymeric matrix that can host a variety of aerobic and anaerobic micro-organisms acting alone or in combination. Their accumulation can lead to the weakening of the bond and possibly the attacking of the tooth by caries.^{3,7} The method of ligation of the archwire is an additional factor of importance for plaque retention. Roughness of the composite surface predisposes to rapid attachment and growth of oral micro-organisms.⁸ The objective of the study was to determine the sites of plaque accumulation and to compare the plaque accumulated with metal and self-ligating orthodontic brackets in order to clarify which bracket type had a higher plaque retaining capacity and to assess accumulation of plaque at the base of bracket, where excess of composite resin is present.

Materials and Methods

Patients (12-25 years) participated in this investigation. Selection criteria were based on the patient's required

orthodontic treatment, including extraction of four premolars and fixed appliances. An informed consent form was signed by the patient/parent before the investigation. The patients were selected from the outpatient block, Department of Orthodontics, and Dentofacial Orthopedics.

A total of 80 teeth premolars were extracted in 20 orthodontic patients and divided into two groups - Group A and Group B with four subgroups in each group. According to the time elapsing between bonding and extraction, one is the control group (teeth without bonded bracket), and others were test groups (teeth with bonded brackets). Each group consists of ten samples.

Group A: Mesh back edgewise metal brackets.

- Subgroup A1: Non-bonded extracted premolar
- Subgroup A2: Bonded metal bracket extracted premolar (1-week group)
- Subgroup A3: Bonded metal bracket extracted premolar (2-week group)
- Subgroup A4: Bonded metal bracket extracted premolar (3-week group)

Group B: Self-ligating brackets

- Subgroup B1: Non-bonded extracted premolar
- Subgroup B2: Bonded self-ligating bracket extracted premolar (1-week group)
- Subgroup B3: Bonded self-ligating bracket extracted premolar (2-week group)
- Subgroup B4: Bonded self-ligating brackets extracted premolar (3-week group)

The mid-buccal surface of all designated teeth is polished with non-fluoridated pumice and acid etched for 30 s (Phosphoric Acid Gel Etchant 37.5%), water rinsed for a minimum of 20 s and dried thoroughly to produce a white frosted appearance of the enamel surface. Before bracket placement, the enamel surface is covered by a thin uniform coat of primer on the tooth surface to be bonded (Primer, Transbond XT 3M). Mesh-backed edgewise Metal (Figure 1) (Victory, 3M Unitek) and self-ligating brackets (Figure 2) (Smart Clip, 3M Unitek) were



Figure 1: Metal brackets bonded on premolars with steel ligatures.

bonded on the buccal surfaces using composite (Transbond XT, 3M). The brackets were adjusted into a position and excess composite was removed with an explorer, explore the light for 20 s. Steel ligature wires were used to ligate metal brackets. One tooth was extracted at each session. All patients were advised to maintain normal dietary and oral hygiene habits. No professional prophylactic care is given and none of the patients used any mouth rinse.

Teeth were extracted 1, 2, and 3 weeks after bracket bonding. Teeth were luxated with a small straight elevator and removed with premolar forceps, which were engaged subgingivally, so as to avoid dislodging the bracket and associated plaque accumulations.

Immediately after extraction, the premolars were rinsed in water to remove blood and debris. Plaque attached to the buccal surfaces was disclosed using plaque disclosing agent (Alpha two). The teeth were immersed in fixative containing 4% formaldehyde and 1% glutaraldehyde in phosphate buffer for 24 h, followed by 0.1 M phosphate buffer for 12 h.⁹

The root and lingual part of the crown were dissected using a high-speed bur under copious irrigation. The specimens are then dehydrated in graded alcohol, desiccated by critical point drying. The specimens were mounted on aluminum stubs and sputter coated with gold prior to scanning electron microscopy (SEM) examination. SEM was operated at an accelerating voltage of 15 kV.

Plaque composition was assessed using SEM. Based on morphological characteristics the bacteria were categorized as cocci, rods, and filaments. In addition, corn-cob formation (coaggregation of filament and coccoid cells) was noted. Assessment of plaque morphology included the mid-buccal area on the excess resin, gingival to the different orthodontic brackets.

Statistical methods

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on mean \pm standard deviation (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5% level of significance. Fisher Exact



Figure 2: Self-ligating brackets bonded on premolars

test has been used to find the significance of study parameters on the categorical scale between two or more groups.

Results

All the extracted premolars were photographed after the buccal surface were stained using Plaque disclosing agent (Alpha plac) (Figure 3). Plaque composition was assessed using SEM at lower ($\times 3600$) (Figure 4) and higher magnification ($\times 5000$) (Figure 5).

To compare the presence of cocci in metal and self-ligating brackets, 4×4 Fisher exact test was carried out. Results showed that the presence of cocci is more and plenty in metal brackets ($P < 0.001^{**}$). Very few colonies of cocci is associated with self-ligating brackets ($P < 0.001^{**}$) (Table 1). Also comparing the presence of rods in metal and self-ligating brackets showed that the presence of rods is more in metal brackets ($P = 0.008^{**}$). Very few rods is associated with Self-ligating brackets ($P < 0.001^{**}$) (Table 2).

Further comparing the presence of filaments and Corn cob formation in metal and self-ligating brackets. Results showed

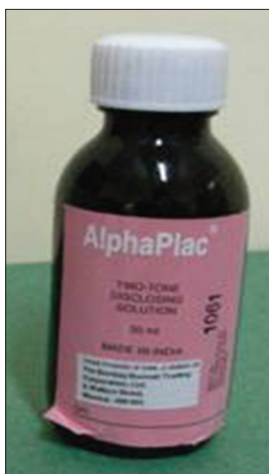


Figure 3: Plaque disclosing agent (Alpha Plac).

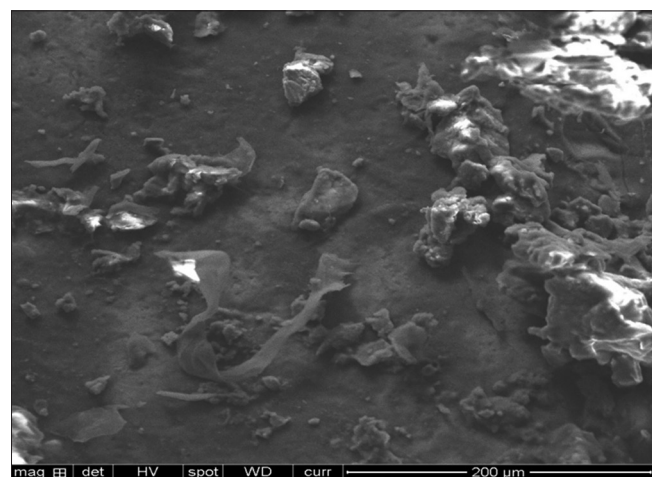


Figure 4: Scanning electron microscopy photograph of cocci, rods, and filaments at $\times 3600$ magnification.

that very few filaments and corn-cob formation is associated with Self-ligating brackets ($P < 0.001^{**}$) (Table 3).

Discussion

Early bonding systems consisted of brackets welded onto bands bonded to enamel with zinc phosphate cement. Apart

Table 1: Comparison of presence of cocci in metal and self-ligating brackets.

Cocci	Metal brackets (%)	Self-ligating brackets (%)	P value
Very few	8 (20.0)	23 (57.5)	<0.001**
Few	13 (32.5)	15 (37.5)	0.913
More	11 (27.5)	0	<0.001**
Plenty	8 (20.0)	0	0.003**
Total	40 (100.0)	40 (100.0)	-

** Statistically significant

Table 2: Comparison of presence of rods in metal and self-ligating brackets.

Rods	Metal brackets (%)	Self-ligating brackets (%)	P value
Very few	16 (40.0)	24 (60.0)	<0.001**
Few	14 (35.0)	14 (35.0)	0.912*
More	8 (20.0)	1 (2.5)	0.008**
Plenty	2 (5.0)	1 (2.5)	0.870
Total	40 (100.0)	40 (100.0)	-

** Statistically significant

Table 3: Comparison of presence of filaments and corn-cobs in metal and self-ligating brackets.

Filaments and corn-cobs	Metal brackets (%)	Self-ligating brackets (%)	P value
Very few	22 (55.0)	26 (65.0)	<0.001**
Few	5 (12.5)	10 (25.0)	0.148
More	13 (32.5)	2 (5.0)	0.172
Plenty	0	2 (5.0)	0.772
Total	40 (40.0)	40 (100.0)	-

** Statistically significant

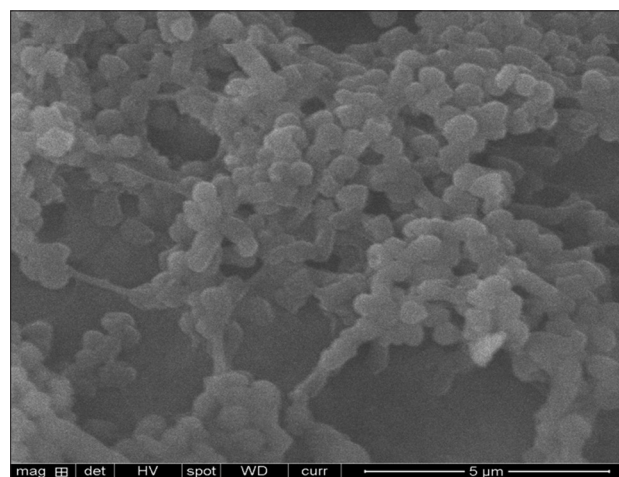


Figure 5: Scanning electron microscopy photograph of cocci, rods, and filaments seen at $\times 5000$ magnification.

from esthetic considerations, this approach presented other serious disadvantages.

Therefore, the need was clear to formulate an alternative procedure that would provide retention of the brackets to tooth enamel without the before-mentioned drawbacks of brackets welded to bands. Hence, direct resin bonding of orthodontic attachments offers many advantages when compared to conventional banding.

Various orthodontic bracket systems have evolved such as gold, metal, plastic, ceramic, and self-ligating brackets. In particular, metallic orthodontic brackets have been found to induce specific changes in the buccal environment such as decreased *H_p*, increased accumulation and elevated *S. mutans* colonization.^{10,11} The placement of ligatures on conventional brackets is time-consuming and has the potential for increased microbial activity in orthodontic practices. Hence, self-ligating brackets which are ligatureless bracket utilize a permanently installed movable component to entrap the archwire and have many advantages over the conventional elastomeric brackets.

Despite the widespread use of fixed orthodontic appliances, little scientific evidence is available on the microbial implications of the different bracket systems *in vivo*. In the light of the above information, no definite conclusion can be drawn about which bracket material has the least plaque retaining capacity. In this investigation, metal and self-ligating brackets were used to compare the plaque retention in the brackets in a fair way, in which the metal brackets were ligated with steel ligatures and self-ligating brackets because this was the most comparable to a clinical situation.

As the bonding composite has a color similar to the enamel surface, it is difficult to detect the residual composite clinically, especially gingival to the bracket base. A SEM technique was chosen for assessing bacterial colonization, as it is a rapid and convenient means of screening microbial samples for major morphotypes. SEM provides a large depth of focus that allows a wide area of the specimen surface to be examined, and it offers a 3D view of a superficial layer of bacterial colonization. Limitation in the use of SEM is the inability to identify specific species; therefore, microorganisms are classified on a morphological basis.¹²⁻¹⁴

Zachrisson and Brobakken showed plaque index were higher in patients wearing mesh back brackets bonded with highly filled resin. Mesh back bracket has less tendency of plaque accumulation than perforated type of brackets.⁷ In the present study, mesh back consisting of metal and self-ligating brackets is used, in which increased plaque retention was associated with metal brackets ligated with steel ligatures and less in self-ligating brackets. This study concurs with theirs as far as mesh back bracket was concerned and no perforated brackets were used.

Within 1 week after the placement of the bracket and during the maintenance of normal oral hygiene, the surface of excess composite gingival to the bracket base was almost completely covered by a thick layer of bacteria, while the enamel surface gingival to the composites revealed a monolayer of bacteria.¹⁴ The same difference in distribution was seen at 2 and 3 weeks in metal brackets and self-ligating brackets. This finding confirms that excess composite around the bracket base along with attachments is an obvious predisposing factor for plaque development due to its rough surface. In the present study, it was observed that excess composite around the metal and self-ligating brackets base is an obvious predisposing factor for plaque development due to its rough surface and variable thickness.

Elastomeric ring and ligature wire are the two commonly used techniques for tying archwires. Based on the results of Forsberg *et al.* revealed that the use of elastomeric rings increases microbial accumulation on the tooth surfaces adjacent to bracket areas, leading to the development of dental caries and gingivitis.¹⁵ In contrast, Tukkahraman *et al.* found no significant differences in the numbers of microorganisms from teeth ligated using similar techniques, with either elastomeric rings or steel ligature wires. Therefore the present *in vivo* study is not in lines with the previous studies as we saw increased microbial adhesion with metal brackets ligated steel ligation and less in self-ligating brackets because the methodology used by Forsberg *et al.* and Tukkahram *et al.* was different.

Pellegrini *et al.* showed that self-ligating brackets promote less retention of oral bacteria, including streptococci, compared with elastomeric orthodontic brackets.¹⁶ The present *in vivo* study concurs with theirs as far the less plaque retention in self-ligating brackets compared with stainless steel ligated metal brackets.

Investigation by van Gastel *et al.* showed orthodontic brackets serve as different loci for biofilm formation. In this study, brackets ligated with an elastomeric ring did not per se show higher bacterial counts compared with self-ligating brackets. SEM images with several enlargement factors revealed remarkable irregularities on the interfaces between different parts of the self-ligating brackets, which might have led to increased biofilm formation on the self-ligating brackets.¹⁷ In the present study, bacterial count was less in self-ligating bracket compared to the metal bracket ligated with a steel ligature. This study is not in accordance with the present study as results from this *in vitro* study cannot be extrapolated to an *in vivo* setting of the present study and also surface characteristics of self-ligating brackets used in the present study.

Excess composite around the bracket base is the critical site of plaque accumulation due to its rough surface texture. The setting shrinkage gap along its periphery is also a predisposing factor for bacterial accumulation. This study highlights the

importance of removing excess composite around the bracket base during bonding and also plaque retention, which is relatively higher in metal brackets with steel ligatures and self-ligating brackets. Further improvement of composite bonding materials and of application technique is needed to reduce the plaque accumulation and consequent development of white spot lesions. Method of ligation is also an important consideration while ligating the orthodontic brackets apart from self-ligating brackets depending on the oral hygiene of the patient. Bracket design of the self-ligating brackets may also contribute to plaque retention.

Conclusion

1. The results of this in vivo study concluded that higher retention of plaque in metal brackets ligated with steel ligatures and comparatively less in self-ligating brackets.
2. Excess composite around the bracket base is the critical site of plaque accumulation associated with fixed appliances due to its rough surface texture.
3. Study shows that unless patients receive specific instructions on appropriate home care, plaque will form on bonded teeth within 1 week after bonding in metal and self-ligating brackets.

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