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# Evaluation of Microbial Adhesion on the Critical Surface of Different Brackets - An Ex Vivo Study

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## ABSTRACT

**Introduction:** The present study was done to evaluate microbial adhesion on the critical surface of different brackets.

**Objectives:** Evaluation of microbial adhesion on the critical surface of different brackets.

**Methods:** The study was conducted on ten different types of commercially available orthodontic brackets. They were categorized into Group 1 and Group 2. Twenty-five MBT (0.22×0.28 slot) upper right premolar brackets were tested in each group. All the brackets were tested without any kind of ligation. CFU was calculated in both groups.

**Results:** Adhesion of aerobic bacteria to Group I brackets are statistically significant ( $p < 0.001$ ). Adhesion of aerobic bacteria to Group II brackets was statistically significant ( $p < 0.001$ ). Adhesion of anaerobic bacteria to Group I was not statistically significant ( $p > 0.05$ ). Adhesion of anaerobes to Group II brackets was statistically significant ( $p < 0.001$ ). Adhesion of anaerobic bacteria is higher in Group II E (46.60) followed by Group II D (42.00), Group II C (37.20), Group IIB (28.04), Group IIA (28.20). The comparison of adhesion of aerobic and anaerobic bacteria to Group I brackets was not statistically significant in Group I B, IC ( $P > 0.05$ ). Statistically, significant correlation found between Group IA, ID, IE brackets towards affinity of aerobic and anaerobic bacteria. Adhesion of aerobic and anaerobic bacteria to Group II brackets were statistically significant ( $p < 0.001$ ).

**Conclusion:** On comparison of aerobes and anaerobes present study was found that significant correlation between adhesion of aerobes to Group I & II brackets whereas no significant correlation found between adhesion of aerobes & anaerobes to Group IB & IC brackets.

**Key Words:** Adhesion, Aerobes, Brackets, Critical surface, Microbes

## INTRODUCTION

The number of patients seeking orthodontic treatment has increased greatly over the last decades. Majority of the patients seek Orthodontic treatment to improve their dentofacial esthetics and some get treated for medical and dental reasons.<sup>1</sup> The orthodontist has to avoid any inadvertent damage to the patient's periodontium or the teeth.<sup>2</sup> Bacterial accumulation on fixed orthodontic appliances can lead to decalcification of the teeth or irreversible damage to the periodontium.<sup>3</sup>

It has been studied by various analysis that fixed orthodontic appliance can lead to an increase in the number of retentive sites for plaque accumulation. This in turn increases with poor oral hygiene.<sup>4</sup> Patients should be counselled about the impor-

tance of maintaining good oral hygiene, especially when the fixed appliance is in place. More importantly, the orthodontist should judiciously choose the right brand of brackets, the one which will cause the lowest bacterial adhesion.<sup>5</sup> The prevalence in choice of brackets varies from patients to patients. The important concern for Orthodontists is to prevent the accumulation of plaque to improve oral hygiene.<sup>6</sup>

For most bacteria in the oral cavity, microbial adhesion to the non-shedding surfaces is the only way to survive. The variability in the design and material of orthodontic brackets may influence plaque adhesion and hence gingival disease.<sup>7</sup> The use of metallic brackets leads to decrease in PH Levels increasing Plaque accumulation and elevated bacterial colonization. Orthodontic brackets are available

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as tooth coloured (plastic or ceramic) or metallic (stainless steel, titanium, or gold).<sup>8</sup> The surface characteristics (roughness and surface free energy (SFE)) of the brackets performance an important role in decreasing friction and plaque (biofilm) formation. Micro- and nanoscale roughness of these brackets can enable initial bacterial adhesion. Even though the surfaces of recently placed brackets are smoother, there can be variations in the surface roughness and SFE during the sequence of orthodontic treatment.<sup>9</sup>

The variability in the design and material of orthodontic brackets may influence plaque adhesion and hence gingival disease.<sup>10</sup> Although a large number of studies have shown a shift in microbial populations in the presence of orthodontic fixed appliances, limited information is available as to which bracket material would be less prone to adhesion of bacterial species and plaque accumulation.<sup>11</sup> The present study was done to evaluate microbial adhesion on the critical surface of different brackets.

## MATERIALS AND METHODS

The study was conducted on ten different types of commercially available orthodontic brackets. They were categorized into Group 1 and Group 2. Twenty-five MBT (0.22×0.28 slot) upper right premolar brackets were tested in each group. All the brackets were tested without any kind of ligation.

Inclusion criteria were patients who were refrained from eating, drinking, and brushing for at least 2 hours before saliva collection, patients with no acute dental caries or periodontal lesions and patients without any pathological lesion.

Exclusion criteria were patients with dental caries or periodontal lesions and patients with any pathological lesion.

The supragingival plaque of two patients wearing fully bonded appliances was collected directly employing sterile curettes before the start of the experiment and was transferred into a sterile flip-capped vial containing 2 ml Reduced transport fluid (RTF). Saliva was collected from two patients, who had refrained from eating, drinking, and brushing for at least 2 hours before saliva collection. Per patient, 5 ml of unstimulated whole saliva was collected in chilled sterile vials by the spitting method.<sup>12</sup>

The brackets were placed randomly in a polyurethane box on a grid with an inter bracket distance of 10 mm. Before the brackets were placed, the floor of the box was roughened with a diamond-coated bur at the exact places to bond the brackets, in such a manner that these areas were completely covered by the bracket bases. The brackets then were bonded with composite bonding material (Transbond XT; 3M Unitek, Monrovia, Calif).

The composite was applied to the bracket base to cover the entire mesh, and the bracket was pressed firmly onto the box.

The excessive adhesive was removed around the brackets. Then the composite was light-cured (3M Unitek, Monrovia, Calif) for 20 seconds from both sides.<sup>13</sup>

The dental plaque and saliva were added to 1 litre of Brain Heart Infusion (BHI), resulting in a concentration of  $8.0 \times 10^3$  CFU aerobe/ml and  $1.4 \times 10^4$  CFU anaerobe/ml. The BHI medium containing bacteria and saliva of the orthodontic patients were inserted into the container in which the brackets were carried and were incubated in an incubator for 72 hours at 37°C (Figures 1 and 2).

The experimental setting then was washed with saline two times to remove all non-adherent bacteria. The brackets were removed randomly with sterile pliers and were transferred into a flip-capped vial containing 1 ml of the pre-reduced transport medium (RTF) and coded. The code used was not revealed, leading to a blind microbiological analysis. The samples then were homogenized by vortexing for 30 seconds and were processed in less than 15 minutes by preparing serial 10-fold dilutions in RTF.

Dilutions of  $10^{-3}$  to  $10^{-5}$  were plated in duplicate employing a spiral plater onto nonselective BHI agar. After 7 days of anaerobic and 3 days of aerobic incubation at 37°C, the total numbers of anaerobic and aerobic CFU (Colony-forming Units) were counted. From these data, the colony-forming units (CFU) ratio (CFU aerobe/CFU anaerobe) was also calculated.

## RESULTS

Table 1 shows the different brackets used in the study. Table 2 shows that adhesion of aerobic bacteria to Group I brackets are statistically significant ( $p < 0.001$ ). The adhesion of aerobic bacteria to Group I brackets are higher in Group IE (43.68), followed by ID (29.68), IC (27.80), IB(19.60), IA (14.84).

**Table 1: Different brackets used in the study**

	Subgroups	Brackets	Quantity
Group I	IA	3M Unitek-Gemini	25
	IB	Dentaurum-Equilibrium-2	25
	IC	Ormco-Mini diamond	25
	ID	American orthodontics-Mini Master Series	25
	IE	Forestadent-Micro Sprint	25
Group II	IIA	Orthox	25
	IIB	Ocean	25
	IIC	Desires	25
	IID	Welcare	25
	IIE	Metro orthodontics	25

**Table 2: CFU aerobes in Group 1**

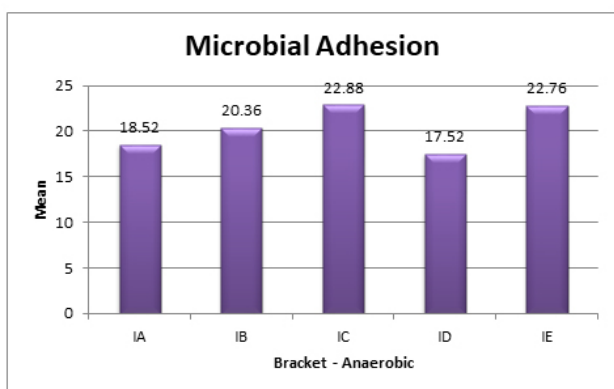
Aerobic – Bracket types Group I	N	Mean	SD	SE	ANOVA	p
I-A	25	14.84	4.78	0.957		
I-B	25	19.60	7.88	1.577		
I-C	25	27.80	13.62	2.724	16.87	0.001**
I-D	25	29.68	13.29	2.658		
I-E	25	43.68	21.40	4.281		
Total	125	27.12	16.54	1.480		

Table 3 shows adhesion of aerobic bacteria to Group II brackets were statistically significant (p<0.001). Adhesion of aerobic bacteria are higher in II E (75.84), followed by IID (64.84), IIC (60.24), IIB (48.20) and IIA (41.92).

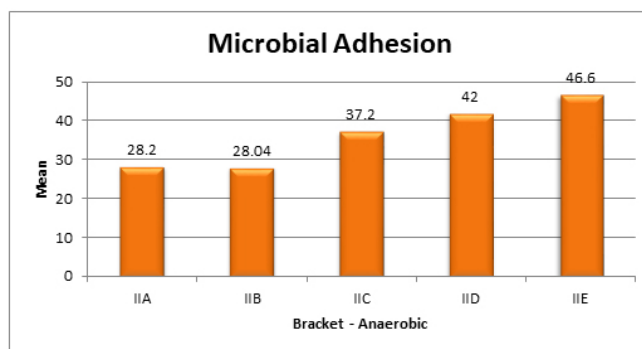
**Table 3: CFU aerobes in Group II**

Aerobic – Bracket types Group II	N	Mean	SD	SE	ANOVA	P
II-A	25	41.92	3.96	0.791		
II-B	25	48.20	7.77	1.555		
II-C	25	60.24	6.27	1.253	63.75	0.001**
II-D	25	64.84	11.09	2.217		
II-E	25	75.84	10.81	2.162		
Total	125	58.21	14.66	1.311		

Figure 1 shows that adhesion of anaerobic bacteria to Group I were not statistically significant (p>0.05). Figure 2 shows that adhesion of anaerobes to Group II brackets was statistically significant (p<0.001). Adhesion of anaerobic bacteria is higher in Group II E (46.60) followed by Group II D (42.00), Group II C (37.20), Group IIB (28.04), Group IIA (28.20).



**Figure 1:** CFU anaerobes in Group I.

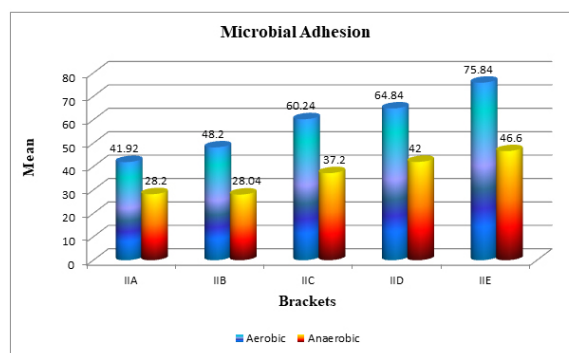


**Figure 2:** CFU anaerobes in Group II.

**Table 4: Comparison of CFU between aerobes & anaerobes in Group I**

Bracket types- Group I	Microbial Adhesion	N	Mean	SD	SE	t	P
I-A	Aerobic	25	14.84	4.78	0.96	2.26	0.028*
	Anaerobic	25	18.52	6.59	1.32		
I-B	Aerobic	25	19.60	7.88	1.58	0.32	0.750
	Anaerobic	25	20.36	8.87	1.77		
I-C	Aerobic	25	27.80	13.62	2.72	1.53	0.134
	Anaerobic	25	22.88	8.62	1.72		
I-D	Aerobic	25	29.68	13.29	2.66	3.93	0.001**
	Anaerobic	25	17.52	7.92	1.58		
I-E	Aerobic	25	43.68	21.40	4.28	4.59	0.001**
	Anaerobic	25	22.76	7.79	1.56		

Table 4 shows that comparison of adhesion of aerobic and anaerobic bacteria to Group I brackets were not statistically significant in Group I B, IC (P>0.05). Statistically, significant correlation found between Group IA, ID, IE brackets towards affinity of aerobic and anaerobic bacteria. Figure 3 shows that adhesion of aerobic and anaerobic bacteria to Group II brackets was statistically significant (p<0.001).



**Figure 3:** Comparison of CFU between aerobes & anaerobes in Group II.

## DISCUSSION

Fixed orthodontic appliances are a significant challenge to the patient concerning maintaining good oral hygiene and avoiding or minimizing the decalcification of enamel during treatment.<sup>12</sup> The decalcification around orthodontic brackets was a common problem and also a potential risk for orthodontic treatment, especially in patients with poor oral hygiene.<sup>13</sup>

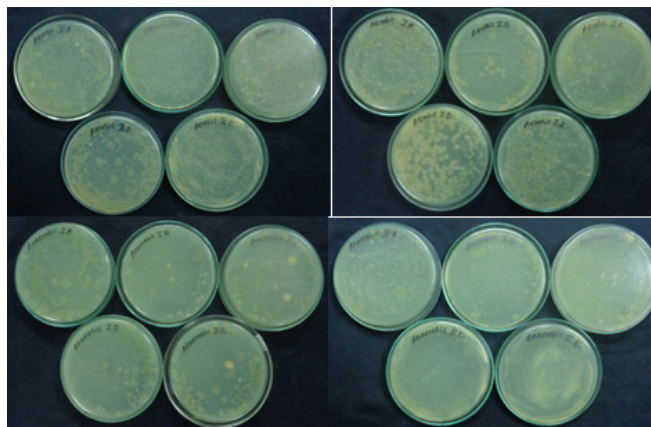
The components of fixed orthodontic appliances create new retention areas that are suitable for bacterial colonization and lead to an increase in the number of microorganisms. Zachrisson et al.,<sup>14</sup> have used clinical parameters as a reference—plaque index, gingival index, probing depth, and bone loss involving periodontal tissues—around orthodontic appliances. However, little information was available on the microbiologic comparisons with the different types of commonly used orthodontic bracket types. Since the advent of increased orthodontic treatment for adult patients, the use of different types of commercially available brackets has become increasingly popular.<sup>15</sup>

Manufacturers usually provide information about the physical properties of the materials, but often fail to include information about their antimicrobial properties. This brings about the need to address questions regarding microbial adherence and biofilm development. The present study was conducted on 10 different types of commercially available fixed orthodontic brackets. They were categorized into Group I (5 different types) & Group II (5 different types). Saliva and plaque was collected from two patients. The brackets were bonded in a polyurethane box on a grid with inter bracket distance of 10 mm.

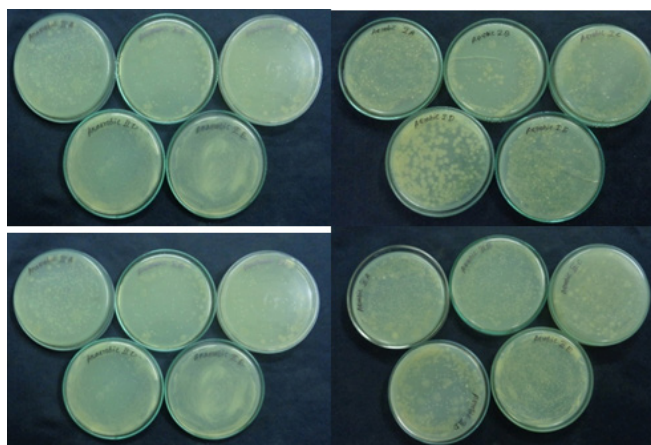
In this present study, adhesion of aerobic bacteria to Group I brackets are statistically significant ( $p < 0.001$ ). The adhesion of aerobic bacteria to Group I brackets are higher in Group IE (43.68), followed by ID (29.68), IC (27.80), IB (19.60), IA (14.84), whereas adhesion of aerobic bacteria to Group II brackets was also statistically significant ( $p < 0.001$ ). Adhesion was higher in IIE (75.84), followed by IID (64.84), IIC (60.24), IIB (48.20) and IIA (41.92). Adhesion of anaerobic bacteria to Group I was not statistically significant ( $p > 0.05$ ). Adhesion of anaerobes to Group II brackets was statistically significant ( $p < 0.001$ ). Adhesion was higher in Group II E (46.60) followed by Group IID (42.00), Group II C (37.20), Group IIB (28.04), Group IIA (28.20).

In this present study, comparison of adhesion of aerobic and anaerobic bacteria to group I brackets were not statistically significant in Group IB, IC ( $P > 0.05$ ), whereas statistically significant correlation was found between Group IA, ID, IE brackets towards affinity of aerobic and anaerobic bacteria. In this present study, Comparison of adhesion of aerobes & anaerobes to group I & II, brackets were statistically significant ( $p < 0.001$ ).

Walker et al.,<sup>16</sup> found that the presence of saliva in the medium was necessary to give adhesion of the oral bacteria to the bracket surfaces. The saliva of the patient donating the dental plaque was used so that an optimal interaction would occur between the salivary components and the microorganisms; this which was recognized as the primary step in biofilm formation and associated diseases.



**Figure 4:** Group I Aerobic and Anaerobic Colonies on the Brain Heart Infusion (BHI) Agar.



**Figure 5:** Group II Aerobic and Anaerobic Colonies on the Brain Heart Infusion (BHI) Agar.

Direct comparisons of results, however, between the different studies must be made with care and must be taken into consideration the different methodologies used to examine the interaction between hard surfaces and bacteria. The adhesion of bacteria on brackets would seem to be more complicated, in a situation like the oral cavity where interactions between the salivary pellicle, many different bacteria, and bracket's surface characteristics take place than the one examined in vitro.<sup>17</sup>

These factors should always be kept in mind when performing adhesion experiments, whether it is brackets or other material. The data obtained from in vitro studies are difficult to

directly apply to the clinical situation, because the raw materials and the bracket fabrication procedures are variable, according to the manufacturers.<sup>18</sup> Besides, the adhesion amount of oral bacteria can be significantly influenced by the morphology of the bracket base. Future studies with bracket raw materials are required to accurately compare the adhesion of oral bacteria according to bracket types. Application of these studies into clinical practice needs extensive clinical studies. Until now, only a few reports have described microbial alterations after placement of different bracket systems in vivo. Future clinical studies of the oral health and microflora between patients wearing different types of brackets would help determine any difference of clinical importance in the plaque composition and the cariogenic effect of each type of bracket on the oral health of the orthodontic patient.<sup>19</sup>

## CONCLUSION

Results of this study indicate major differences between the different bracket types in terms of adhesion of supragingival aerobic as well as anaerobic oral microbes over different bracket types. On comparison of aerobes and anaerobes present study was found that significant correlation between adhesion of aerobes to Group I & II brackets whereas no significant correlation found between adhesion of aerobes & anaerobes to Group IB & IC brackets.

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**Authors contribution**

1. Dr. Shanmuga sundaram R- Data collection
2. Dr. Sunil Sunny- Investigation
3. Dr. J. Aruna- Data collection
4. Dr. Ranganathan- Manuscript writing
5. Dr. Varsha Sobha- Analysis
6. Dr. Arthy Priya- editing

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