ORIGINAL ARTICLE

Adherence of Streptococcus Mutans and Lactobacillus on Different Orthodontic Brackets: An In-Vivo Study.

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ABSTRACT

Objective: To investigate the effect of different types of orthodontic brackets on growth and adherence of microorganisms in oral flora Streptococcus mutansand Lactobacillus.

Method: Invivo study a total of 10 patients for fixed orthodontic treatment were selected and bonding of the brackets was done randomly in each quadrant with split mouth technique giving a total sample of 40. Sample were divided into 4 equal groups based on bracket type & use: Mini Master Stainless steel brackets, Silkon Plus ™ Plastic brackets, Radiance Plus™ Ceramic brackets,Titatinumorthos. After one month, the brackets were then debonded and collected in a sterile medium for microbiological study. The microbial colonies were counted and all data was analyzed through one way ANOVA and Tukey-Kramer test.

Result:The data analysis showed there was no significant difference in adherence of Lactobacillus and Streptococcus mutans on titanium and plastic brackets whereas, significant difference present in retention of Lactobacillus among all the other groups of bracket.

Conclusion:The titanium brackets have the highest microbial adhesion due to its surface roughness and there was also a close relevance in adhesion of S.mutans to plastic and ceramic bracket groups. For patients primarily at increased risk of dental caries or periodontal diseases, or both, microbial adhesion could be a significant determining factor in the selection of orthodontic brackets.

Keywords: Fixed orthodontic appliance, lactobacillus, streptococcus mutans, colony forming unit.

INTRODUCTION

Oral environment provides the ideal condition for complex microbiota in which they co-exist in a balanced state with their host. When there is imbalance in this micro-flora it may lead to a pathologic condition or disease. There is an increased colonization of microorganisms during the fixed appliance therapy that is largely due to structural irregularities, hence, accumulation of plaque is more in patients with Fixed Orthodontic Appliance (FOA) and are at higher risk for enamel demineralisation which aggravates the effect of pre-existing incipient carious lesions. [1]

Electrostatic and hydrophobic interactions are mostly due to affinity of bacteria to solid surfaces. During the process of adherence of bacteria to hard surfaces it has been seen that the physiochemical properties of bacteria as well as of solid surface contribute as mediators, the composition as well as rate of

salivary secretion affects rate of bacterial adherence. [2]

Oral environmental alterations such as reduced levels of pH, increased plaque accumulation and increased levels of *streptococcus mutans* colonization have been found to induce due to metallic orthodontic brackets. Studies on possible differences in adherence of bacteria and initial affinity of bacteria on metal, ceramic and plastic brackets over the time have been found to be inconclusive. [3]

Table I: Mean distribution and standard deviation of *lactobacillus* and *streptococcus mutans* adherence on different orthodontic brackets.

Brackets	Group A		Group B		Group C		Group D	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
L.bacillus	501.30	95.73	855.30	84.81	680.90	80.98	923.80	174.55
S.mutans	600.90	96.34	864.80	91.78	751.80	124.18	948.90	170.43

MATERIAL AND METHODS

This study was undertaken to investigate the effect of different types of FOA on the growth and adherence of *Streptococcus mutans* and *Lactobacillus* in oral flora. This study was started after getting approval by the Ethics Committee of People's College of Dental and Research Centre, People's University (approval no.: 2015ORT02). The study was done using following inclusion criteria, patients with bimaxillary protrusion or undergoing all 4 first premolar extractions were selected with good oral hygiene, healthy gums with no signs of redness, oedema or bleeding gums, no antibacterial mouthwash or antibiotics also no professional cleaning aids were included during the course of the study. Patients with pre-existing periodontal conditions, pregnancy or having any systemic diseases, were excluded in the study. Subjects with informed consent and following the inclusion and exclusion criteria were recruited in the study.

This was an in vivo study in which total of 10 patients, planned for orthodontic treatment were selected and brackets were bonded randomly in each quadrant with split mouth technique giving a total sample of 40. Bracket sample will be divided into 4 equal groups based on bracket type & use i.e. Group A Mini Master Stainless steel brackets (American orthodontics, Sheboygan WI, USA. MBT 0.022 x 0.028),Group B :SilkonPlus™ plastic brackets (American Orthodontics, Sheboygan, WI, U.S.A MBT 0.022 x 0.028),Group C : Radiance Plus™ ceramic brackets (American Orthodontics, Sheboygan, WI, U.S.A. MBT 0.022 x 0.028), Group D :TitatinumOrthos (Ormco Corporation West Collins Avenue Orange, CA MBT 0.022x 0.028. Prior to bonding, thorough oral prophylaxis was carried out in all the patients. Bonding was carried out by using Transbond XT (3M Unitek, CA, U.S.A.) light cure composite resin from single operator to avoid inter- operator bias in all the four groups. Routine oral hygiene instructions were given to the patients after bonding the brackets on the labial surface of first premolars one type of bracket in each quadrant. Patient was recalled after one month and the brackets were debonded; bracket sample was collected with the help of debonding plier by debonding it from the labial surface of teeth and were taken in sterile medium for further microbiological study.Under sterile environment, 100µl suspension of the debonded bracket sample were used for spread plate method for suspected bacteria in each sample using micropipette. The sample was spread over the prepared petri plates containing selective media for enumeration of microbial species. Using sterile L-shaped plastic spreader. The plates were then incubated for 24-48 hours at 37˚C in a bacteriological incubator. The counting of microbial colony was done after incubation using digital colony counter. Since the main samples were not diluted by any factor, so the bacterial count was regarded as "Standard Plate Count (SPC)." Two different agar plates were prepared in the laboratory

1. Mitis Salivariousbacitracin (MS-Agar) media
2. MRS (deMan- Rogosa Sharpe) agar media

Mitis Salivarious bacitracin agar media supplemented with 1% potassium tellurite and bacitracin was used as selective media specifically for the growth of *Streptococcus Mutans* and MRS (De-Mann Rogosa Sharpe) agar media as the selective media specifically was used for the isolation of *Lactobacillus species*.

Sodium acetate 5g/l was added to inhibit the growth of other bacteria. The isolation of *S. mutans* and *lactobacillus* colonies was done.

The possible observation on the selective media were as **follows:**

On MRS Agar media: small pin point dark blue/black convex colonies were *S.mutans.*

On MS-Agar media: Creamy white smelly and convex colonies on chocolate brown media will describe the presence of *Lactobacillus.*

All the data was recorded on excel sheet and subjected to statistical analysis. Data was analysed using SPSS software (Statistical Package for the Social Sciences,version 22.0; Chicago, III USA), for the purpose of data analysis, p (probability) value ≤ 0.05 was taken to be statistically significant and ≤ 0.01 was considered statistically highly significant.

RESULTS

Mean distribution and standard deviation of colony forming unit of *Lactobacillus* on orthodontic brackets of different material are as follows. Group A 501.30 ± 95.73, Group B 855.30 ± 84.81, Group C 680.90 ± 80.98, Group D 923.8 ± 174.55 (Table I and Fig.1).

Mean distribution and standard deviation of colony forming unit of *Streptococcus mutans* of various groups (A, B, C, D) are600.90 ± 96.34, 864.80 ± 91.78, 751.80 ± 124.18, 948.90 ± 170.43 respectively (Table I and Figure1).

Figure.1 Comparative graph of Mean distribution of *lactobacillus and streptococcus mutans* adherence on different types of orthodontic brackets

One way ANOVA test (Table II) was performed to compare the mean value of colony forming unit count of the two microbes i.e.Streptococcus mutans and lactobacillus for all the four groups group of brackets. All the parameters shows a statistically significant difference between the stainless steel brackets, plastic bracket, ceramic brackets and titanium brackets. Adhesion of *Streptococcus mutans*, to the four types of brackets shows P<0.0001, f=14.5. Adhesion of *Lactobacillus* to the four types of orthodontic brackets shows P<0.000 f= 26.86.

Post hoc analyses specifically, Tukey's HSD tests were conducted on all possible pair wise contrasts. Group A, Group B, Group C and Group D were compared between each other on the basis of adhesion of *lactobacillus* for all the four group of orthodontic brackets which shows significant difference (P<0.05).

Table II: One wayannova (P –values, F- values)

Brackets	*L. bacillus*		*S. mutans*	
Group comparison	F-value	P-value	F-value	P-value
	26.862	.000**	14.594	.000**

(**p value is highly significant at p<0.00001)

For *Lactobacillus*, (Table III) Group B shows significant difference with Group C (P=0.009). Group B shows highly significant difference with Group A (P=0.000), Group C shows significant difference with Group A (P=0.007), Group D shows highly significant difference with Group A and Group C (P=0.000) and Group D shows no significant difference with Group B (P=0.553).

For Streptococcus Mutans, (Table IV) Group B shows highly significant difference with Group A (P=0.000) and Group B shows no significant difference with Group C (P=0.197), Group C shows significant difference with Group A (P=0.49), Group D shows highly significant difference with Group A and Group C (P = 0.000), Group D shows no significant difference with group B (P = 0.443).

Adherence of *Lactobacillus* on orthodontic brackets from the comparative analysis show that group D, i.e. titanium brackets present highly significant difference with group A, i.e. stainless steel brackets and group C, i.e. ceramic brackets and shows no significant difference with Group B, i.e. plastic brackets. The comparative statistical analysis show that the adherence of S.mutans was found to be most on the group D brackets i.e. Titanium brackets, whereas there was a significant difference between adherence of S.mutans on ceramic and stainless steel brackets andno significant difference between the adherence of S.mutans on titanium and plastic brackets.

DISCUSSION

Orthodontic appliances frequently encroach on the gingival sulcus and act as an obstacle for maintaining the oral hygiene. Increase in inflammation is noted immediately after placement

of fixed orthodontic appliances. The level of oral hygiene during treatment has a direct influence on periodontal status. Even with excellent oral hygiene, the majority of patients usually develop moderate gingivitis within few months after placement of the appliances. These changes are generally transient and are reversible with no permanent damage to the periodontal tissues. [4, 5] The purpose of our study wasto assess the adherence of cariogenic bacteria *Streptococcus mutans*(SM) and *Lactobacillus* (LB)on commercially available orthodontic bracket system made of different material in clinical situation to clarify which bracket type has a higher retaining capacity of these bacteria. 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. Organism that was found to be recently adhering to orthodontic brackets was *S mutans* (in vivo). [6] In addition, brackets were found to induce specific changes in the oral environment such as decreased pH, increased plaque accumulation, and elevated *S mutans*colonization. This indicates that orthodontic brackets could be a potential risk for enamel demineralization. Many different types of orthodontic brackets are commercially available. Their mechanical properties, including frictional resistance and bond strength, and their morphologic nature and structure have been studied extensively. It is a common belief that plaque formation during treatment with fixed appliances is mainly attributable to the complexity of the bracket design. Additionally, nonspecific or specific interactions between the microbial and bracket material surface occur, especially at early stages of biofilm formation. Cumulation of ***Streptococcus mutans***and***Lactobacillus*** on the enamel surrounding the orthodontic attachments is common in fixed orthodontic therapy, Because of its well documented role in pathogenesis of dental and enamel demineralization. [7] *S. mutans* along with glycosyl transferase degrades sucrose to make insoluble glucans. These insoluble glucans also attach to the tooth surface, providing ideal sites for oral bacteria to inhabit. The resulting complex of glucan and various bacteria then creates an oral biofilm which is the mature stage of dental plaque. Due to high cariogenic property of these microbes they have been selected for the present study. As plaque accumulates, acidic compounds such as fructose and other fatty acids degrade the enamel surface of the teeth through a process known as dental caries. [8] Although a number of studies have demonstrated the viability of SM and LB on removal orthopedic appliances, little is known about their survival on fixed orthodontic appliances. [7, 9, 10]

Table III: Group wise comparative analysis of *lactobacillus*. Post hock analysis (Tukey's HSD)

			Mean difference	Std error	P value
L. bacillus	Group B	Group A	354.00*	51.66	.000**
	Group B	Group C	174.40*	51.66	.009** *
	Group C	Group A	179.60*	51.66	.007** *
	Group D	Group A	422.50*	51.66	.000**
	Group D	Group B	68.50	51.66	.553
	Group D	Group C	242.90*	51.66	.000**

(*the mean difference is significant at the 0.05 level, **p value is highly significant at p<0.00001,* p value is significant at p<0.05, p value is insignificant at p>0.05)**

Agar media[3] was used in this study as this is easily available, less technique sensitive and more economical. Various techniques are available for microbiological analysis of microorganisms like Checker board technique developed by DNA probe technique [6] fluorescent dye radioisotope [2], radioactive labelling [11], scanning electron microscopy [8] etc which have proven to be more sensitive, reliable but are very expensive. The results of our in-vivo study was found to be in concordance with numerous other studies demonstrating the adhesion of SM and LB was weaker on metallic (s.s.) than on plastic or ceramic brackets indicating that metallic brackets had a lower potential for bacterial accumulation than plastic and ceramic brackets. Two surface characteristics of material are known to influence the adhesion of bacteria; surface roughness and surface free energy. [12] A material with a rough surface or a high surface free energy attracts more bacteria to its surface than that with a smooth surface or a low surface free energy. Higher adhesion to the titanium brackets was may be due to very rough surface. Low adherence on s.s. brackets were noted which may be because of the reason that saliva coating reduces the surface free energy of underlying metal brackets. **Fournier et al** [2] found that adhesion of *S.mutans* is weaker on metal than plastic and ceramic brackets. Result of our study was also congruent with the findings of study performed by **Van gastel et al** [13] concluded orthodontic brackets serve as different loci for biofilm formation. Significant differences between the different bracket types in terms of biofilm formation were found. The adherences of the micro-organism were less with metallic brackets when compared to ceramic brackets.**Brusca et al** [7] performed a similar study in which they assessed micro-organism (SM and candida species) on different bracket system using electron microscopy and

demonstrated their adhesion of microorganisms together varied according to the bracket materials with composite >ceramic > metallic.

Reddy R.P et al [16]quantitatively evaluated the adhesion of cariogenic ***Streptococci*** (*Streptococci mutans and Streptococci sobrinus*) to orthodontic metal and ceramic brackets, with respect to incubation time and the saliva coating among the brackets used; metal brackets had highest number of microorganisms adhered compared to ceramic brackets. An evaluation of the microbial profile on metallic and ceramic bracket materials was carried out by**Anhoury et al** [6]using SM and LB species and stated to have no significant difference or no obvious pattern of bacterial colonization between the different brackets with respect to the caries inducing micro-organism species counts.

Table IV: Group wise comparative analysis of Streptococcus*mutans*. Post hock analysis (tukey's HSD)

			Mean difference	Std error	P value
S.mutans	Group B	Group A	263.90*	55.75537	.000**
	Group B	Group C	113.00	55.75537	.197
	Group C	Group A	150.90*	55.75537	.049***
	Group D	Group A	348.00*	55.75537	.000**
	Group D	Group B	84.10	55.75537	.443
	Group D	Group C	197.10*	55.75537	.006**

(*the mean difference is significant at the 0.05 level., **p value is highly significant at p<0.00001, *** p value is significant at p<0.05, p value is insignificant at p>0.05)

The outcome of our study came forth to be very much alike the above mentioned study. Quantitative determination of the cariogenic streptococci (SM and S.sorbinus) varied pattern of adhesion was on various brackets monocrystalline sapphire, polycrystalline alumina, stainless steel, plastic, and titanium brackets was observed by **Ahn SJ et al.** [12] No variation in the result was observed on comparing with the present study (Titanium >plastic > ceramic).**Rammohan SN et al** [8]quantified the adherence of *S.mutans*and *C.albicans* on s.s, plastic, ceramic, titanium and gold brackets with scanning electron microscopy demonstrating the highest adherence of microorganisms to ceramic brackets and least to stainless steel supporting the results of our study.Eliades et al (1995) [14]suggested that the metal bracket has increased bacterial adhesion because of their high surface energy compared with that of plastic and ceramic brackets.

In an experimental study done by**Ahn et al** [11]**,** he analyzed the effects of bracket pellicles on the adherence of Streptococcus gordonii and Streptococcus mutans binding affinity of SM increased with extended incubation time. The interaction effects between saliva-coating and incubation times, and between bracket types and incubation times. Binding affinity for MCS brackets was increased less than for s.s, plastic brackets by extended incubation time. **Saloom H.F et al** [15]studied oral strains of S. mutans and Candida albicans using biochemical test, microbial suspensions was prepared to do the tests of each microorganism. It was demonstrated that the appliance with high esthetic appearance, sapphire brackets and coated arch wire, showed the least adherence of *S. mutans*and *Candida albicans* in comparison to other appliances with less aesthetic and more metal components.

Considering the limitations of the study additional iological studies using split mouth design would further help delineate any possible relationship between bracket composition and microbial flora that colonize them. Clinical studies of dental and gingival health between patients with each bracket type would help determine any possible clinical significance of these subtle differences in plaque composition of different bracket types. Also many other parameters could be taken into the account of this experiment like form, size and osition of the brackets, presence or absence of gingival hook, prophylaxis and longer study duration etc.

CONCLUSION

- In this study we found that the adhesion amount of both the microbes was highest on titanium brackets due to its surface roughness and least on stainless steel brackets.
  No significant difference in adherence of *S.mutans* to plastic and ceramic bracket was found.
- A significant increase of lactobacillus and *S. mutans*adhesion was noted with plastic brackets.
- Significant difference was observed in adherence of Lactobacillus on titanium and stainless steel brackets; plastic and ceramic brackets; titanium and ceramic brackets.

- Microbial adhesion could be a crucial determining factor in the selection of orthodontic brackets, for patients having increased risk of dental caries or periodontal diseases, or both.

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