

An In Vivo Comparison of Bacterial Colonization with Orthodontic Bracket System

Neha Agarwal^{1*} Umang Agarwal² Swati Katiyar³ Shubhi Gupta⁴

¹Senior Lecturer, Department of Orthodontics, Maharana Pratap Dental College, Mandhana, Kanpur, UP, India.

²Senior Lecturer, Department of Oral and Maxillofacial Surgery, Maharana Pratap Dental College, Mandhana, Kanpur, UP, India.

³Senior Lecturer, Department of Prosthodontics, Rama Dental College, Kanpur, UP, India.

⁴Private Practitioner, Dehradun, Uttarakhand, India.

ABSTRACT

Aim: The objective of this in vivo study was to compare the amount of bacterial colonization associated with metal, self-ligating and ceramic orthodontic brackets.

Materials and Method: The study was done on 30 orthodontic patients who were randomly divided in to three groups. Group I bonded with metal brackets wire ligated with steel ligature, Group II bonded with self-ligating brackets and Group III bonded using ceramic brackets wire ligated with elastomeric module. Amount of bacterial colonization was evaluated from right of the maxillary dental arch at day 1 and at day 21, the aerobic and anaerobic bacterial count was then compared.

Result: ANOVA test for anaerobic and aerobic log bacterial count showed significant difference between group I, group II and group III observations at 5% level of significance at day 21.

Conclusion: The result of this in vivo study concluded that higher bacterial colonization was associated with ceramic brackets ligated with elastomeric modules followed by metal brackets ligated with steel ligatures and comparatively less microbial growth was observed in self-ligating brackets.

Keywords: Bacterial, Orthodontic brackets, Orthodontics.

INTRODUCTION

The placement of orthodontic appliances create a favourable environment for the accumulation of a microbiota and food residues¹. The development of dental plaque has been associated with several environmental and individual factors including diet composition, oral hygiene, fluoride exposure, the quality of saliva, the composition of the oral microflora, and immune factors. Fixed or removable orthodontic appliances also impede the maintenance of

oral hygiene, resulting in plaque accumulation². Adhesion of microorganisms to surfaces is a result of electrostatic interactions and van der Waals forces³. Patients undergoing fixed orthodontic appliance treatment have elevated levels of *S. mutans*, *Candida* species and Enterobacteriaceae. Undisturbed supragingival plaque initiates gingival inflammation further leading to gingivitis and gingival hyperplasia⁴. There is clear evidence that fixed appliances induce continual accumulation and retention of bacterial plaque⁵ and initiates gingival inflammation.



Received: Jan. 17, 2015; Accepted: Apr. 16, 2015

*Correspondence Dr. Neha Agarwal.

Department of Orthodontics, Maharana Pratap Dental College, Mandhana, Kanpur, UP, India.

Email: nehaa_239@yahoo.co.in



Fig 1: Robertson Cooked meat media.



Fig 4: Identification of anaerobic bacteria based on metronidazole.



Fig 2: Brain Heart Infusion Blood Agar.

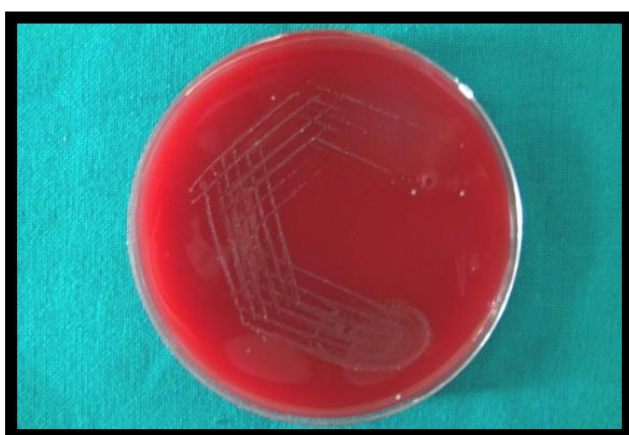


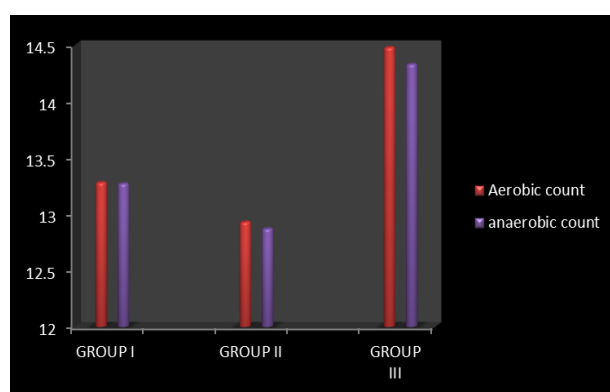
Fig 3: α Hemolytic Streptococcus Viridians group.

Table 1: Comparison of log bacterial growth on day 1 (anaerobic and aerobic).

	Group I	Group II	Group III	P value
Anaerobic count	10.61±0.22	10.50±0.33	10.48±0.32	0.558
Aerobic count	10.45±0.52	10.41±0.40	10.29±0.29	0.664

Table 2: Comparison of log bacterial growth on day 21 (anaerobic and aerobic).

	Group I	Group II	Group III	P value
Anaerobic count	13.27±0.14	12.87±0.28	14.33±0.39	<0.001**
Aerobic count	13.28±0.21	12.93±0.43	14.48±0.33	<0.001**



Graph 1: Comparison of log aerobic and anaerobic bacterial growth at day 21.

The composition of dental plaque determined by dark-field microscopy showed significant shifts in the test sites after banding. Changes consisted of an increase in the percentage of spirochetes, motile rods, filaments and fusiforms; conversely, a decrease in cocci was noted⁶.

Composites used as a direct bonding adhesive have a polymeric matrix that can host a variety of aerobic and anaerobic microorganisms acting alone or in combination. Their accumulation leads to the weakening of the bond and possibly the attacking of the tooth by caries^{7,8}. Roughness of the composite surface predisposes to rapid attachment and growth of oral microorganisms⁹.

In a study by Fournier, Payant and Bouclin¹⁰ adherence of streptococcus mutans to the orthodontic brackets, it was proved that saliva coating on bracket surface causes a decreased affinity for streptococcus mutans for all the products. A study for microbiological evaluation of elastomeric chain was done by Casaccia, Gomes, Alviano et al¹¹. In this study the surface of elastomeric chains of different manufacturers were used to verify the presence of pathogenic microorganisms at the moment of unpacking and analyze a possible inhibitory effect of the elastomeric chain when exposed to microorganisms of the oral cavity.

It has been proved in various studies that different materials used in fixed mechanotherapy has different rate of microbial growth. In this in vivo investigation metal, self-ligating and ceramic brackets were used to compare the bacterial growth. The purpose of this study was to compare the in vivo bacterial colonization associated with three types of brackets system used.

MATERIALS AND METHOD

Thirty patients undergoing orthodontic treatment were selected for this study from Department of Orthodontics and Dentofacial Orthopedics.

The following inclusion criteria were used for patient selection:

- a. Patients in age group of 11-25 years.
- b. Patient undergoing fixed orthodontic treatment with brackets on their anterior teeth and bands on their molars.

The following exclusion criteria were used for patient selection:

- a. Presence of decalcification of teeth.
- b. Presence of anterior composites.

The patients were randomly divided in to three groups. Group I bonding was done using metal brackets wire ligated with stainless steel ligature wire, Group II patients bonding was done using self-ligating brackets and Group III bonding was done using ceramic brackets wire ligated with elastomeric module. Bonding was done using composite (Transbond XT, 3M). The patients were instructed to brush once in the morning before breakfast and once in the evening before bed time. They were instructed to brush a minimum of three minutes to ensure thorough brushing. The patients were asked to thoroughly rinse with water after every meal.

Method of Sample collection

Samples were collected on two visits, labelled as day 1 and day 21.

- 1) On day1 after the oral prophylaxis, the swab from the buccal surface of second premolar was collected from the right side using sterile endodontic paper points placed on the surface of the tooth for 30 seconds. Immediately upon removing, the paper points were transferred to Robertson cooked meat media (RCM), used as the transport media (Figure 1).
- 2) After the swab was collected, bonding was done.
- 3) On day 21 bracket was collected from the maxillary right second premolar side of the dental arch (Group I patients metal bracket along with steel ligature, Group II patients self-ligating bracket and Group III patients ceramic bracket along with elastomeric

module) and transferred to RCM, to be carried to microbiological lab.

Culture Procedure

- 1) The RCM was incubated overnight and from the overnight culture an aerobic subculture, an anaerobic subculture with metronidazole, and a dilution was performed for colony count.
- 2) For culture on the solid media a well was prepared using an inoculation loop separately on Blood Agar (BA) for aerobic culture and on Brain Heart Infusion (BHI) blood agar plate (Figure 2) for anaerobic culture. A metronidazole disc was firmly pressed on the prepared well and BHI agar plate was immediately packed in gas pack jar creating an atmosphere of 95% hydrogen and 5% carbon dioxide for incubation. Incubation was done for 5 days at 37 degree C in an incubator.
- 3) Sample inoculated in 25 microliter saline was immediately dispersed using a vortex at maximal setting for 60 seconds. The dispersed sample was labelled as X and was serially diluted making 2x, 4x, 8x dilutions with the help of micropipette for obtaining a countable growth of colonies on BA and BHI blood agar plate.
- 4) 10 microliters of each dilution was spread on separate BHI blood agar plate using a sterile inoculation loop and incubated in an anaerobic environment for 5 days, and 10 microliters of each dilution was spread on separate BA plate using a sterile inoculation loop and incubated in candle jar for 48 hours.

Identification of Bacteria

- A. Aerobic Bacteria: After 48 hours of incubation, colonies were identified on Blood agar plates on the basis of colony characteristics, gram stain, and α hemolysis. *Streptococcus viridians* group (Figure 3) was identified on the basis of α hemolysis around the colonies on blood agar with green discoloration, catalase test, optochin sensitivity and bile solubility.

- B. Anaerobic Bacteria: After 5 days of incubation, colonies were identified on BHI agar plates on the basis of characteristic colony morphology, pigment production and metronidazole disc sensitivity (Figure 4). The presence was further confirmed by gram staining, aero - tolerance test, catalase test and special potency disc sensitivity test (kanamycin, vancomycin, colistin and SPS).

RESULTS

The statistical analysis was carried out using SAS 9.2, SPSS15.0, Stata 10.1, MedCalc 9.0.1 and Systat 12.0. All bacterial counts were converted to log bacterial count for ease of statistical calculations. The mean and standard deviations of the bacterial counts values were calculated for all the groups. Analysis was done in two phases: descriptive and inference. ANOVA test was used to compare the three groups and Paired *t*-test was used to compare the mean anaerobic and aerobic bacterial counts on day 1 and day 21 in each group.

Aerobic and the anaerobic bacterial counts were recorded in the study and the data was obtained. Significant differences were found between day 1 and day 21 (Graph I) observations at 5% level of significance with respect to group I, group II and group III (Tables I and II).

ANOVA test for anaerobic and aerobic log bacterial count (Table I) showed no significant difference between group I, group II and group III observations at 5% level of significance with respect to day 1. On day 21 significant differences between groups I, II and III at 5% level of significance (Table - II) were noted.

DISCUSSION

Primary dental care begins at home. Practicing satisfactory oral hygiene, such as adequate tooth brushing, mouth rinsing, and dental flossing, plays a vital role in maintaining healthy teeth, especially in the orthodontic patients¹². It is a well-known fact that the placement of fixed orthodontic appliances generally hinders good oral hygiene and the appliance component can cause alteration in oral micro flora by reducing pH, increasing affinity of bacteria to the metallic surface because of electrostatic reactions and causing

retention areas for microorganisms. Thus they lead to plaque accumulation around the bracket base².

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 pH, increased accumulation and elevated streptococcus mutans colonization¹³. The placement of ligature on conventional brackets is time consuming and has a potential for increased microbial activity in orthodontic practice. Hence self-ligating brackets which are ligature less brackets utilize a permanently installed movable component to entrap the arch wire and have many advantages over the conventional brackets, such as predictable and very low friction¹⁴ and reduces the risk of precutaneous injury¹⁵.

Elastomeric ring and ligature wire are the two commonly used techniques for tying arch wires. Forsberg et al¹⁶ evaluated microbial colonization of 12 patients treated by fixed orthodontic appliances and reported that the lateral incisor attached to the arch wire with an elastomeric ring exhibited a greater number of microorganisms in the plaque than teeth ligated with steel wire. They also reported a significant increase in the number of *S. mutans* and lactobacilli in the saliva after the insertion of fixed appliances. They recommended that the use of elastomeric ligation rings should be avoided in patients with inadequate oral hygiene because elastomeric ligation rings will significantly increase microbial accumulation on tooth surfaces adjacent to the brackets, leading to a predisposition for the development of dental caries and gingivitis. On the other hand, Sukontapatipark et al⁵ and Turkkahraman² evaluated the microbial colonization of 20 patients. Upper second premolar was selected as the donor site and the sample was collected at three different time intervals. They found no significant difference between both materials regarding microbial contamination. Therefore, the present in vivo study is in line with Forsberg et al as there was increased microbial colonization with ceramic brackets ligated with elastomeric rings followed by metallic brackets ligated with steel ligation and less in self-ligating brackets.

Peter Pellegrini et al showed that self-ligating brackets promote less retention of oral bacteria, including streptococci compared with elastomeric orthodontic brackets¹⁷. The present in vivo study concurs with the above study as far the less microbial colonization is associated with self-ligating brackets as compared to metal and ceramic brackets.

CONCLUSION

The result of this study concluded that higher bacterial colonization was associated with ceramic brackets ligated with elastomeric modules followed with metal brackets ligated with steel ligatures and comparatively less bacterial growth was found in self-ligating brackets.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Owen OW, A study of bacterial counts (lactobacilli) in saliva related to orthodontic appliances; a preliminary report. *Am J Orthod.* 1949;35(9):672-8.
2. Turkkahraman H, Sayin MO, Bozkurt FY, Yetkin Z, Kaya S, Onal S. Archwire ligation techniques, microbial colonization, and periodontal status in orthodontically treated patients. *Angle Orthod.* 2005;75(2):231-6.
3. Christersson CE, Dunford RG, Glantz PO, Baier RE. Effect of critical surface tension on retention of oral microorganism. *Scand J Dent Res.* 1989;97(3):247-56.
4. Magno AF, Enoki C, Ito IY, Matsumoto MA, Faria G, Nelson-Filho P. In-vivo evaluation of the contamination of Super Slick elastomeric rings by *Streptococcus mutans* in orthodontic patients. *Am J Orthod Dentofacial Orthop.* 2008;133(4 Suppl):S104-9.
5. Sukontapatipark W, el-Agroudi MA, Selliseth NJ, Thunold K, Selvig KA. Bacterial colonization associated with fixed orthodontic appliances. A scanning electron microscopy study. *Eur J Orthod.* 2001;23(5):475-84.

6. Huser MC, Baehni PC, Lang R. Effects of orthodontic bands on microbiological and clinical parameters. *Am J Orthod Dentofacial Orthop.* 1990;97(3):213-8.
7. Ulukapi H, Koray F, Efes B. Monitoring the caries risk of orthodontic patients. *Quintessence Int.* 1997;28(1):27-9.
8. Zachrisson BU, Brobakken BO. Clinical comparison of direct versus indirect bonding with different bracket types and adhesives. *Am J Orthod.* 1978;74(1):62-78.
9. Mitchell L. Decalcification during orthodontic treatment with fixed appliances-an overview. *Br J Orthod.* 1992;19(3):199-205.
10. Fournier A, Payant L, Bouclin R. Adherence of streptococcus mutans to orthodontic brackets. *Am J Orthod Dentofacial Orthop.* 1998;114(4):414-7.
11. Rembowski Casaccia G, Gomes JC, Alviano DS, de Oliveira Ruellas AC, Sant' Anna EF. Microbiological evaluation of elastomeric chains. *Angle Orthod.* 2007;77(5):890-3.
12. Lee SJ, Kho HS, Lee SW, Yang WS. Experimental salivary pellicles on the surface of orthodontic materials. *Am J Orthod Dentofacial Orthop.* 2001;119(1):59-66.
13. Eliades T, Viazis AD, Eliades G. Bonding of ceramic brackets to enamel: morphologic and structural considerations. *Am J Orthod Dentofacial Orthop.* 1991;99(4):369-75.
14. Pizzoni L, Ravnholt G, Melsen B. Frictional force related to self ligating bracket. *Eur J Orthod.* 1998;20(3):283-91.
15. McNamara JA Jr, Bagramian RA. Prospective survey of percutaneous injuries in orthodontic assistants. *Am J Orthod Dentofacial Orthop.* 1999;115(1):72-6.
16. Forsberg MC, Brattstrom V, Malmberg E, Nord CE. Ligature Wires and elastomeric rings: two methods of ligation, and their association with microbial colonization of *Streptococcus mutans* and lactobacilli. *Eur J Orthod.* 1991;13(5):416-20.
17. Pellegrini P, Sauerwein R, Finlayson T, McLeod J, Covell DA Jr, Maier T, Machida CA. Plaque retention by self-ligating vs elastomeric orthodontic brackets: quantitative comparison of oral bacteria and detection with adenosine triphosphate-driven bioluminescence. *Am J Orthod Dentofacial Orthop.* 2009;135(4):426.e1-9.

How to cite this article:

Agarwal N, Agarwal U, Katiyar S, Gupta S. An In Vivo Comparison of Bacterial Colonization with Orthodontic Bracket System. *Adv Hum Biol.* 2015;5(2):72-77.