

Microbial colonization around orthodontic ligature ties: An *in-vivo* study

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Abstract

Introduction: Fixed or removable orthodontic appliances impede oral hygiene and result in plaque accumulation. Studies have evaluated the effects of fixed orthodontic appliances on microbial flora and periodontal status, but only a few have evaluated the method of ligation as an additional factor. **Aims and Objectives:** To determine the amount of microbial colonization with different elastomeric ligation techniques. **Material and Methods:** Twenty subjects and a total of five ligature systems divided into five groups were chosen for the split mouth study. Group II and III (super slick ties and slide ligatures) were ligated over the premolars on the right side while Group IV and V (quick stick ties — angulated and easy to fit ties) were ligated over the premolars on the left side in both the arches. Group I (control) or stainless steel ties were made over the cuspids in the same manner. Elastomeric ligatures were removed after a period of 21 days and sent for culture. The amount of microbial colonies was then compared using stereomicroscope. **Results:** The amount of microbial colonization observed was statistically significant ($P < 0.001$) in all the groups. Group III showed the highest microbial colonization while Group II showed the least. **Conclusion:** Super slick elastomeric ligatures were found to have minimal microbial adhesion similar to that of stainless steel.

Key words: Elastomeric ligature, plaque, decalcification

INTRODUCTION

The development of dental plaque has been associated with several environmental and individual factors including diet composition,^[1,2] oral hygiene,^[1] fluoride exposure,^[3] quality of saliva, composition of the oral microflora, and immune factors.^[4,5] Fixed or removable orthodontic appliances also impede the maintenance of oral hygiene, resulting in plaque accumulation.^[5,6] Plaque retention surrounding orthodontic appliances leads to enamel demineralization caused by organic acids produced by bacteria in the dental plaque.^[7,8]

Recently, fluoride-releasing elastomeric modules^[9-11] and chlorhexidine varnish^[12] were suggested for reducing plaque accumulation and decalcification.

Fixed orthodontic appliances create new retention areas, which are suitable for bacterial colonization and lead to an increase in the absolute number and percentage of *Streptococcus mutans* and lactobacilli.^[4,13-24] A lot of studies have evaluated the effect of fixed orthodontic appliances on microbial flora and periodontal status,^[13-15,17,20,22,24-27] but only a few studies evaluated the methods of commercially available elastomeric ligations as an additional factor.^[4,28]

Therefore, the study was conducted to determine the amount of microbial colonization on different elastomeric ligation ties available commercially and assess as to which elastomeric ligature attracts the least microbial flora and fulfills the criteria for hygienic ligatures.

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MATERIALS AND METHODS

Twenty subjects were selected. Informed consent was obtained from all subjects. Inclusion criteria for patient selection included age group from 18 to 25 years; complement of permanent dentition, free of dental plaque, motivated for good oral hygiene and undergoing fixed orthodontic treatment with standardized MBT 0.022 slot (Gemini series, 3M Unitek, Monrovia, California, USA). Subjects with presence of (a) decalcification or increased risk to caries (high caries index), (b) use of antibiotics during the three-month period, and (c) halitosis and poor periodontal status were excluded from the study. Subjects 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 3 min to ensure thorough brushing and rinse with water after every meal. The investigation was designed as a split-mouth study.

A total of five ligature systems divided into five individual groups were chosen for the study [Table 1, Figures 1-4]. Groups II and III were ligated over the premolars on the

right side of the patient both in the upper and lower arch while Groups IV and V were ligated over the premolars on the left side of the patient both in the upper and lower arch. Group I (control) or stainless steel ties were made over the cuspids on either side [Figures 5 and 6].

Elastomeric ligatures were removed from the patient's mouth under aseptic conditions on the 21st day and collected in labeled sterile vials with normal saline and sent for microbiological assessment to quantify the amount of microbial colonization on different elastomeric ligature groups as obtained from the patient's mouth [Figure 7].

Table 1: Commercially available orthodontic ligature ties

Group I (Control Group)	Stainless steel
Group II	Super Slick (TP Orthodontics Inc. La Porte, Indiana, USA)
Group III	Slide ligatures (Leone S.p.A. Via P. a Quaracchi, Sesto Fiorentino, Firenze, Italy)
Group IV	Quick Stick Tie- Angulated Ties (3 M Unitek)
Group V	Easy to Fit Tie (3 M Unitek, Monrovia, California, USA)

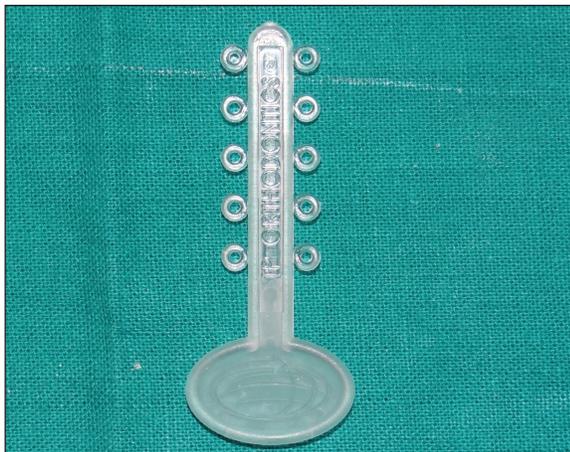


Figure 1: Super slick ligature ties



Figure 2: Slide low friction ligature ties



Figure 3: Quick stick tie — angulated ligature ties

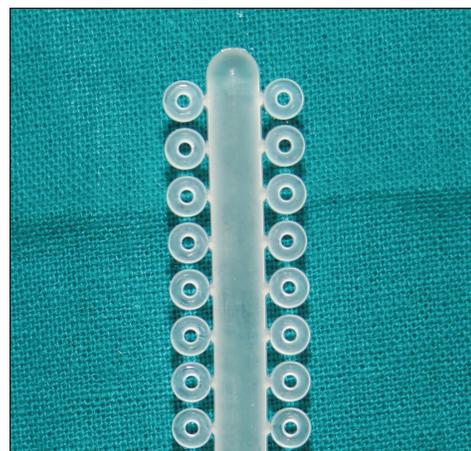


Figure 4: Easy-to-fit tie ligature ties



Figure 5: Ligatures tied on right buccal segment using split mouth technique



Figure 6: Ligatures tied on left buccal segment using split mouth technique



Figure 7: Samples collected in labeled vials with normal saline



Figure 8: *S. aureus* on sheep blood agar



Figure 9: *S. mutans* on sheep on blood agar

Stuart transport medium was used and the samples were sent without delay. Serial 10-fold dilutions of the transport medium with the sample of plaque were prepared to 10^{-4} , and 0.1-ml samples were inoculated on sheep blood agar [Figures 8 and 9], Chocolate agar and Robertson cooked meat broth for number of total bacteria and Mitis – Salivarius agar (Difco Laboratories Inc., Detroit, MI, USA) containing 0.001% Chapman Tellurite solution (Difco), 150 g sucrose, and 3.33 mg bacitracin (Sigma Diagnostics, St. Louis, MO,

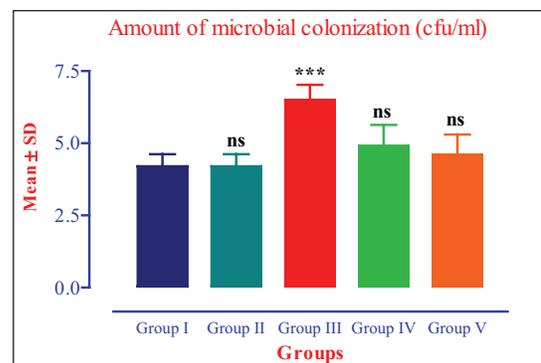


Figure 10: Amount of microbial colonization in the groups (cfu/ml)

USA) per liter agar for number of *S. mutans*. The samples were even inoculated on MacConkey agar (HiMEDIA) for secondary bacterial infections. The agar plates were incubated for 48 h at 37°C in anaerobic jars. Subsequently, colonies were counted under a stereomicroscope. Also, serial 10-fold dilutions were prepared to 10^{-3} , and 0.1-ml samples were inoculated on two Rogosas agar plates for number of lactobacilli. Both plates were incubated for 48 h at 37°C, one plate in aerobic conditions and the other in an anaerobic jar. The number of colonies were

Table 2: Amount of microbial colonization (Mean±SD, n=20) of five groups

Group I	Group II	Group III	Group IV	Group V	F value (4,45 DF)	P value
4.20±0.42 (4-5)	4.20±0.42 (4-5)	6.50±0.53 (6-7)	4.90±0.74 (4-6)	4.60±0.70 (4-6)	27.21	P<0.001

Numbers in parenthesis indicates the range

Table 3: Significance (P value) of mean difference of amount of microbial colonization between the groups by Tukey’s test

Comparisons	Mean difference	q value	P value
Group I vs. Group II	0.00	0.00	>0.05
Group I vs. Group III	2.30	12.60	<0.001
Group I vs. Group IV	0.70	3.83	>0.05
Group I vs. Group V	0.40	2.19	>0.05
Group II vs. Group III	2.30	12.60	<0.001
Group II vs. Group IV	0.70	3.83	>0.05
Group II vs. Group V	0.40	2.19	>0.05
Group III vs. Group IV	1.60	8.76	<0.001
Group III vs. Group V	1.90	10.41	<0.001
Group IV vs. Group V	0.30	1.64	>0.05

then determined under a stereomicroscope. Results are expressed as colony-forming units per milliliter. Groups of different ligature ties were then compared by one-way analysis of variance (ANOVA) and the significance of mean difference between the groups was done by Tukey’s post hoc test. A two-sided ($\alpha = 2$) $P < 0.05$ was considered statistically significant. Analyses were performed on STATISTICA Version 6 for Windows (Statsoft, Inc, Tulsa, Oklahoma, USA).

RESULTS

Statistically significant microbial colonization was observed in all the groups (P -value < 0.001). It was highest for Group III (6.50 + 0.53) whereas Group II showed the least amount of microbial colonization (4.20 + 0.42), which was comparable to Group I (4.20 + 0.42). Group IV (4.90 + 0.74) and Group V (4.60 + 0.70) showed slightly higher microbial colonization as compared to Groups I and II but lesser than Group III [Table 2, Figure 10].

Intergroup comparison showed statistically significant microbial colonization in Group III elastomeric ligature ties when compared to Groups I, II, IV, and V [Table 3].

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 patients.^[8] 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 base.

The literature clearly demonstrates that fixed orthodontic appliances increase plaque accumulation, bacterial colonization, and resultant enamel decalcification.^[13-15,17,20,22,24,26] However, the contribution of ligation materials to this increase has only been evaluated in a few studies.^[4,28] Forsberg *et al.*^[4] evaluated microbial colonization of 12 patients treated by fixed orthodontic appliances and reported that the lateral incisor attached to the archwire 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 al.*^[28] and Turkkahraman^[34] evaluated the microbial colonization of 20 patients. Upper second premolar was selected as the donor site; the sample was collected at three different time intervals. They found no significant difference between both materials regarding microbial contamination.

In this study, maxillary second premolars were selected as the donor site for microbial samples because the posterior teeth are more prone to plaque accumulation also access to cleaning is less in posterior region. Bacterial sampling was performed at day 1 when thorough oral prophylaxis was done and then at day 21, which is equivalent to the average duration between orthodontic appointments. The study was terminated on the third week because longer periods of observation may affect the results as cooperation, motivation for oral hygiene and dietary habits can change.

This study showed highest microbial colonization with Group III or slide low friction elastomeric ligatures when ligated over the area of premolars. These ligatures produce minimal friction owing to a reduced treatment time; therefore strict hygiene instructions with change of ligatures every appointment would solve any gingival inflammations caused by microbial adhesion over these elastic ligature ties. Slide low friction ligature ties are made of polyurethane mix and have a tubular structure that passively secures the archwire beneath to allow it to slide freely. Therefore, because of its design it tends to attract higher colonies of oral microflora.

Group II or super slick ties (SSt) become extremely slippery when wet or moistened in the oral environment. The highly lubricious surface has proven to reduce friction more than 70% during orthodontic treatment. Bacterial adhesion over these ligature ties were seen to be the least and similar to the control group or the stainless steel ligature ties. The polymeric coating that is used on super slick ligatures demonstrates a resistance to bacteria. Previous studies reveal that SSt that the lubricious coating over these elastomeric ligature ties resists bacterial adhesion levels by 90% in six strains of bacteria tested.^[36] Our study was in contrast to the conclusion made by Forsberg et al. who stated that elastomeric ligation should be avoided in patients with poor oral hygiene and that stainless steel ligation should be attempted. Group II or super slick elastomeric ligatures were found to have a similar microbial colonization as that of stainless steel in our study.

Though Groups IV and V, that is, quick stick ties — Angulated (3 M Unitek) and easy-to-fit ties (3 M Unitek), respectively, found a higher microbial colonization than Superslick elastomeric ties but the microbial colonization was significantly lesser than slide low friction elastomeric ligatures.

Results obtained from the present *in-vivo* study performed on the 21st day cannot be extrapolated to the findings that would be observed with long time intervals. Advancements in the manufacturing of currently available elastomeric ligatures have not only reduced the amount of friction but even reduced the amount of microbial adherence over these attachments similar to that of stainless steel ties.

CONCLUSION

- Super slick elastomeric ligature ties showed the least amount of microbial flora adherence which was comparable to control group or the stainless steel ligatures.
- Slide low friction ligatures showed the maximum

adherence to microbial flora owing to its complex tubular design.

- Quick stick ties — angulated ligatures and easy-to-fit ligatures showed a slight increase of microbial flora adherence though it was found to be statistically insignificant.

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