

“MICROBIOLOGICAL FLORA OF HYGIENIC LIGATURES”- AN IN-VIVO STUDY

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ABSTRACT: Fixed or removable orthodontic appliances impede the maintenance of oral hygiene and result in plaque accumulation. Many 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. **MATERIALS AND METHODS:** Subjects for this study was selected on the basis of good oral hygiene. Amount of microbial colonization was evaluated from both right and the left sides of the maxillary dental arch at day 1 that is (after receiving through oral prophylaxis) and then the arch wire ligated with split mouth technique using Super Slick ties (TP Orthodontics) and Dumbell ligatures (Leone) on right side & Aelastixs- Quick stick ties- Angulated ties (3M Unitek) and Aelastixs- Easy to fit Ties (3M Unitek) on left side. Elastomeric ligatures were removed from the patients’ mouth after a period of 7 days and sent for culture of the aerobic and anaerobic bacterial colonies. The amount of microbial colonies was then compared using stereomicroscope. **RESULTS:** The current study revealed that the teeth ligated with elastomeric rings exhibited greater number of aerobic as well as anaerobic microorganisms and the difference was found to statistically significant.

KEYWORDS: Oral hygiene, Split mouth technique

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 the quality of saliva, the 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 lead to enamel demineralization caused by organic acids produced by bacteria in the dental plaque.^{7, 8} Recently, fluoride-releasing elastomeric modules⁹⁻¹¹ and chlorhexidine varnish¹² 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} . So this study was done to determine the amount microbial colonization with different elastomeric ligation techniques available.

AIMS AND OBJECTIVES

- 1) To determine the amount microbial colonization with different elastomeric ligation techniques available.

ORIGINAL ARTICLE

- 2) Assess which elastomeric ligature attracts the least microbial flora and fulfills the criteria for Hygienic ligatures.

MATERIALS AND METHODS: Twenty subjects were selected for the present study. Informed consent was obtained from all subjects.

- Following inclusion criteria were used for patient selection:
 - ✓ Patients of age group 18-25years.
 - ✓ All patients were in the permanent dentition, free of dental plaque, and motivated for good oral hygiene.
 - ✓ Patient undergoing fixed orthodontic treatment with standardized 3M MBT Gemini Series 0.022 slots.
- Following exclusion criteria were used for patient selection:
 - ✓ Presence of decalcification or increased risk to caries (high caries index)
 - ✓ Subjects who had used antibiotics during the three-month period before the study were excluded.
 - ✓ Poor periodontal status.
 - ✓ Halitosis.

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. The investigation was designed as a split-mouth study.



Fig 1&2: Patient in permanent dentition, free of dental plaque, and motivated for good oral hygiene.

A total of five ligature systems divided into five individual groups were chosen for the study. These were as follows:

Group I	Super Slick (TP Orthodontics)
Group II	Dumb bell ligatures (Leone)
Group III	Easy to Tie- Angulated Ties (3 M Unitek)
Group IV	Quick Stick Ties (3 M Unitek)
Group V (Control group)	Stainless steel

Table 1: Samples of elastomeric ties selected for microbial colony assessment.

ORIGINAL ARTICLE



Fig 3: Group I - Super Slick (TP Orthodontics) Fig 4: Group II- Dumb bell ligatures (Leone)

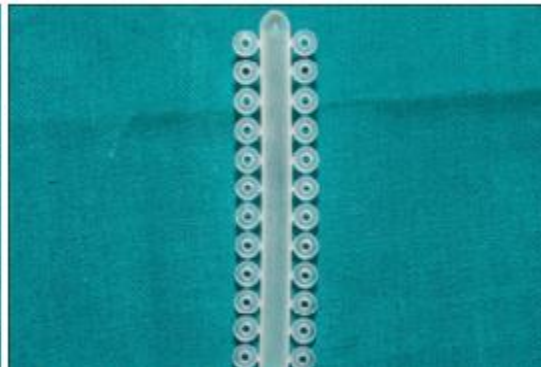


Fig 5: Group III- Easy to Tie- Angulated Tie.
(3 M Unitek)

Fig 6: Group IV - Quick Stick Ties
(3 M Unitek)

Group I and II were ligated over the premolars on the right side of the patient both in the upper and lower arch with stainless steel ties i.e. Group V (Control group) over the cuspids on either side while Group III and IV were ligated over the premolars on the left side of the patient both in the upper and lower arch.



Figure7: Group I and II ligated over the premolars on the right side.



Figure8: Group III and IV ligated over the premolars on the left side.

Elastomeric ligatures were removed from the patient's mouth on the 7th day and collected in labeled sterile vials with normal saline and sent to the department of Microbiology to assess the

ORIGINAL ARTICLE

amount of microbial colonization on different elastomeric ligature groups as obtained from the patients' mouth.



Fig 9&10: Labeled sterile vials which were sent to the Department of Microbiology

The elastomeric sample groups were collected under aseptic conditions. 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, Chocolate agar & 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, USA) per liter agar for number of S mutans. The samples were even inoculated on MacConkey agar (Hi MEDIA) for secondary bacterial infections. The agar plates were incubated for 48 hours 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 hours at 37°C, one plate in aerobic conditions and the other in an anaerobic jar. The number of colonies were then determined under a stereomicroscope. Results are expressed as colony-forming units per milliliter.

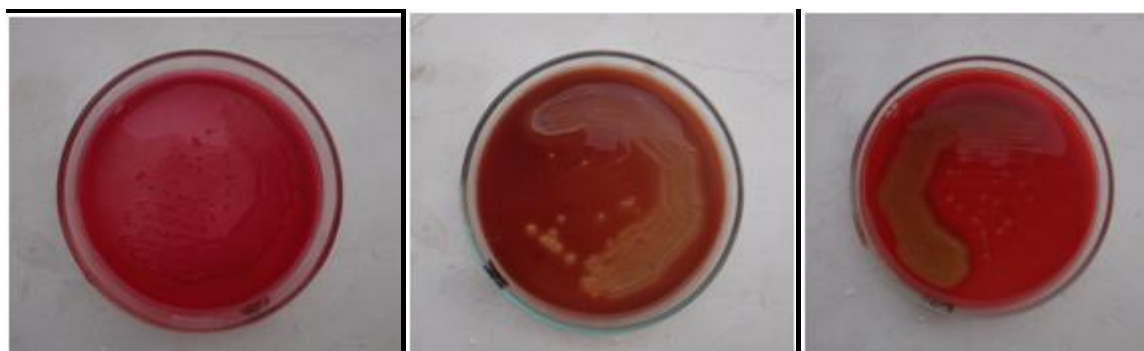


Fig 11: E. coli (Lactose fermenter on Mac Conkey Agar) Fig 12: S. Aureus on Sheep blood Agar Fig 13: S. Mutans on Sheep blood Agar.

RESULTS:

Subjects	Group 1	Group 2	Group 3	Group 4	Group 5
1	10 ⁴ CFU/ml	10 ⁶ CFU/ml	10 ⁴ CFU/ml	10 ⁴ CFU/ml	10 ⁴ CFU/ml
2	10 ⁴ CFU/ml	10 ⁷ CFU/ml	10 ⁵ CFU/ml	10 ⁵ CFU/ml	10 ⁵ CFU/ml
3	10 ⁵ CFU/ml	10 ⁶ CFU/ml	10 ⁴ CFU/ml	10 ⁵ CFU/ml	10 ⁴ CFU/ml
4	10 ⁵ CFU/ml	10 ⁶ CFU/ml	10 ⁵ CFU/ml	10 ⁴ CFU/ml	10 ⁴ CFU/ml
5	10 ⁴ CFU/ml	10 ⁷ CFU/ml	10 ⁵ CFU/ml	10 ⁴ CFU/ml	10 ⁵ CFU/ml
6	10 ⁴ CFU/ml	10 ⁷ CFU/ml	10 ⁵ CFU/ml	10 ⁴ CFU/ml	10 ⁴ CFU/ml
7	10 ⁴ CFU/ml	10 ⁷ CFU/ml	10 ⁶ CFU/ml	10 ⁵ CFU/ml	10 ⁴ CFU/ml
8	10 ⁴ CFU/ml	10 ⁶ CFU/ml	10 ⁶ CFU/ml	10 ⁶ CFU/ml	10 ⁴ CFU/ml
9	10 ⁴ CFU/ml	10 ⁶ CFU/ml	10 ⁵ CFU/ml	10 ⁴ CFU/ml	10 ⁴ CFU/ml
10	10 ⁴ CFU/ml	10 ⁷ CFU/ml	10 ⁴ CFU/ml	10 ⁵ CFU/ml	10 ⁴ CFU/ml
11	10 ⁴ CFU/ml	10 ⁶ CFU/ml	10 ⁷ CFU/ml	10 ⁵ CFU/ml	10 ⁴ CFU/ml
12	10 ⁴ cfu/ml	10 ⁶ CFU/ml	10 ⁶ CFU/ml	10 ⁴ CFU/ml	10 ⁴ CFU/ml
13	10 ⁴ cfu/ml	10 ⁷ CFU/ml	10 ⁶ CFU/ml	10 ⁴ CFU/ml	10 ⁴ CFU/ml
14	10 ⁴ cfu/ml	10 ⁷ CFU/ml	10 ⁷ CFU/ml	10 ⁵ CFU/ml	10 ⁴ CFU/ml
15	10 ⁵ cfu/ml	10 ⁷ CFU/ml	10 ⁶ CFU/ml	10 ⁴ CFU/ml	10 ⁵ CFU/ml
16	10 ⁴ cfu/ml	10 ⁶ CFU/ml	10 ⁵ CFU/ml	10 ⁴ CFU/ml	10 ⁶ CFU/ml
17	10 ⁵ cfu/ml	10 ⁷ CFU/ml	10 ⁴ CFU/ml	10 ⁵ CFU/ml	10 ⁴ CFU/ml
18	10 ⁴ cfu/ml	10 ⁶ CFU/ml	10 ⁵ CFU/ml	10 ⁴ CFU/ml	10 ⁵ CFU/ml
19	10 ⁵ cfu/ml	10 ⁶ CFU/ml	10 ⁵ CFU/ml	10 ⁴ CFU/ml	10 ⁶ CFU/ml
20	10 ⁴ cfu/ml	10 ⁷ CFU/ml	10 ⁵ CFU/ml	10 ⁴ CFU/ml	10 ⁴ CFU/ml

Table 2: A total of 20 subjects evaluated for the amount of microbial flora adhered over each group that is calculated in colonies forming unit per ml on the 7th day after their placement.

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 patients⁸. 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 al⁴ 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

ORIGINAL ARTICLE

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 al²⁸ and Turkkahraman³⁴ 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 through 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.

The result of the current study revealed that the teeth ligated with elastomeric rings exhibited greater number of aerobic as well as anaerobic microorganisms and the difference was found to statistically significant. This result is in accordance with the study of Forsberg et al⁴ but is in contrast with the study of Turkkahraman et al²⁸ who found statistically not significant difference between elastomers and ligatures. A feasible explanation may be due to the difference in the study design i.e. in the present study at the 21 day the elastomeric ring and the ligature was cultured unlike the previous study were the swab from the labial surface of the tooth was taken and in this study anaerobic as well as aerobic culture was done whereas in previous study specifically the growth of streptococcus mutants and lactobacillus was evaluated.

CONCLUSION OF THE STUDY: After analyzing the findings of the present study it was concluded that Super Slick elastomeric ligature ties showed the least amount of microbial flora adherence comparable to stainless steel ties. Slide low friction ligatures showed the maximum adherence to microbial flora owing to its complex tubular design.

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ORIGINAL ARTICLE

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ORIGINAL ARTICLE

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