Microbial colonisation on elastomeric modules during orthodontic treatment - an in vivo study

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Abstract

Purpose: The aim of this study was to determine the presence or absence of Streptococcus Mutans and Lactobacilli from the elastomeric modules and to study its extent at different intervals of time during orthodontic treatment.

Methods: In this prospective clinical study, 30 patients were selected for arch wire ligation with commonly used elastomeric modules (3M Unitek). One elastic module collected at regular intervals of at the pre-treatment (T0), at end of 1st week (T1), 5th week (T2) &8th week (T3) forisolation and assessment of the microbial growth. These collected modules were cultured & incubated to check for the presence or absence of Streptococcus Mutans & Lactobacilli and numbers of colonies were counted at each interval. Results obtained were subjected to Wilcoxon matched Paired Test to compare the growth statistically.

Results: The study revealed that there is presence of Streptococcus Mutans and Lactobacilli during orthodontic treatment. With increased duration of time, there was progressive increase in the colonization of these microbes on the elastomeric modules.

Conclusion: On placement of elastomeric modules for ligation and its evaluation at different intervals of time i.e. 1st, 5th & 8th week, there is statistically significant rise in the microbial colonization of Streptococcus mutans & Lactobacilli during orthodontic treatment. Thus, on examination of elastomeric modules over a scheduled period of time microorganisms were isolated which indicates that patients undergoing fixed mechanotherapy and ligated with elastomeric modules should exercise maximum oral hygiene with oral hygiene adjunctive aids other than routine brushing and the orthodontist should take appropriate measures to avoid any iatrogenic damage and alteration of the oral environment.

Keywords: Elastomeric Modules, Microbial Colonization, Orthodontic Treatment.

Introduction

The placement of a fixed appliance often impedes the maintenance of good oral hygiene for orthodontic patient. Plaque retention surrounding orthodontic appliances leads to enamel demineralization caused by organic acids produced by bacteria in the dental plaque(1). Fixed orthodontic appliances create new retention areas, which are suitable for bacterial colonization and lead to an increase in the absolute number of Streptococcus Mutans & Lactobacilli.(2)

The drawback of these fixed attachments are accumulation and retention of plaque which constitutes a high risk of white spot lesion and enamel demineralization if the patient does not maintain the oral hygiene or use appropriate oral hygiene aids(4).

Numbers of studies have evaluated the effect of fixed orthodontic treatment on microbial flora but only a few studies have evaluated microbial colonization on the elastomeric modules at different intervals of time during orthodontic treatment. Therefore, this study has helped us to determine the presence and extent of Streptococcus Mutans & Lactobacilli on elastomeric modules at different time intervals during orthodontic treatment and its colonization during the course of orthodontic treatment.

Methods

30 patients(16 males & 14 females) undergoing orthodontic treatment in the Department of Orthodontics and Dentofacial Orthopedics of Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belgaum, Karnataka were selected. Informed consent was obtained from all patients. Ethical clearance has been obtained for the study.

Inclusion criteria:

1. Patients in the age group of 12-25 years.
2. Patients in permanent dentition.

Exclusion criteria:

1. Subjects who had used antibiotics during and three month period prior to the study.
2. Patients having history of smoking or history of any periodontal disease.
3. Patients with any systemic disease

Sample collected was grouped as under:
Group 1-Control (T0)
Group 2-at the end of 1st week (T1)
Group 3- at the end of 5thweek (T2)
Group 4-at the end of 8th week (T3)

Patients selected were undergoing fixed orthodontic treatment with standardized 3MUnitek MBT Gemini Series 0.022 slots. Oral prophylaxis was

carried in all patients. Standard oral hygiene instructions were given to all the patients.Arch wires were ligated with commonly used3M Unite kelastomeric modules (Fig. 1 & 2) and the patients were followed regularly over a period of 8weeks. Samples of the modules in the as received state from the company were also checked for the presence or absence of Streptococcus mutans and Lactobacilli. Further, samples of the elastomeric modules were collected (Fig. 3) randomly from the teeth (one posterior and one anterior region) at the end of 1st (T1), 5th (T2) & 8th week (T3) during orthodontic treatment.

Elastomeric ligatures were removed from the patient's mouth under aseptic conditions at the end of 1st week and collected in labelled sterile air tight vials of Transport media (Fig. 4).

After collection of modules in the transport media, it was sent to Department of Microbiology at the Maratha Mandal NGH Institue of Dental Sciences' laboratory for isolation and quantitative assessment of the microbial growth of Streptococcus Mutans & Lactobacilli on the elastic module. The suspension was further diluted to $10^{-(3)}$ in sterile saline.

To evaluate for the presence of Streptococcus mutans, the sample was cultured on selective media Mitissalivaris agar (MSA) and incubated for 37°C for 48 hours under $C0_2$jar (Fig. 5). To confirm the presence of Streptococcus mutans, Biochemical test of sugar fermentation was done by checking the growth on Phenol red sorbitol broth (Fig. 6). To check for presence of Lactobacilli, Rogosa agar was used and incubated anaerobically at 37°C for 48-72hours (Fig. 7). Subsequently, the colonies were counted with a colony counter (Fig. 8). The same procedure was repeated at the end of 5th week & 8th week.

Results

The results of microbial colonies were counted and described with frequencies at pre-treatment (T0), at the end of 1st week (T1), at the end of 5th week (T2), at the end of 8th week (T3). The readings obtained are presented in (Table 1 and graph 1 & 2).

The study revealed that there is presence of Streptococcus Mutans and Lactobacilli during orthodontic treatment at all the intervals of time during orthodontic treatment. With increased duration of time, there was progressive increase in the colonization of these microbes on the elastomeric modules.

The results were analysed statistically with the Wilcoxon non parametric test at 5% significance level (Table 2, 3).

Discussion

The literature clearly demonstrates that fixed orthodontic appliances increase plaque accumulation, bacterial colonisation and resultant enamel decalcification.[2]

Bonded orthodontic brackets hinder access for good oral hygiene and create microbial shelters, resulting in the accumulation of plaque. The appliance architecture — specifically, the archwire ligation method - is an additional factor in influencing bacterial colonization.

Archwire ligation can be done with 0.020 to 0.036mm stainless steel ligature wires or elastomeric modules. Elastomeric modules were first introduced to orthodontics nearly three decades ago and have gained almost universal acceptance by the orthodontic profession.

Orthodontists prefer elastomeric rings as they are time saving, patient friendly, easy for application; have an aesthetic appearance, potential for fluoride release.

Patients with orthodontic appliances apart from providing new retention areas of bacterial colonization will also experience oral ecologic changes such as low salivary pH, increased retention of food particles which may lead to increase in levels of salivary S mutans [4,5]. Oral hygiene is a significant prophylactic program in receiving good orthodontic treatment. Decrease in pH, increased affinity of bacteria to the metallic surface because of electrostatic reactions are the main factor occurred due to fixed orthodontic appliance component causing alteration in oral microflora.[6,7]

Some studies have shown that the elastomeric ring exhibit bacterial plaque on its surface, with a higher number of microorganisms than can be verified on tooth surfaces because of its rough surface and the absorption properties of this material[2,7,8]. The design and surface characteristic of both orthodontic attachment and composite may influence plaque retention.

The prevention of enamel decalcification during fixed appliance treatment remains a challenge to the orthodontist[9,10]. Since individuals who undergo orthodontic treatment, have elevated levels of Streptococcus mutans[11], the control of plaque is fundamental in the prevention of caries and periodontitis[12]. Unless patients receive specific instructions on appropriate home care, abundant plaque may form on bonded teeth within one week[7]. In addition to brackets and orthodontic bands, other accessories can lead to the accumulation of microorganisms. Each material can induce specific alterations in the oral environment, such as pH reduction and the prolonged accumulation of dental biofilm[13]. Elastomeric rings may lead to an increased retention of plaque and risk of gingival bleeding compared to steel ligatures Türkkahraman et al. also observed that gingival tissue adjacent to teeth ligated with elastomeric rings was more prone to bleeding. Steinberg and Eyal[14] observed that modules have demonstrated high adsorption capability of whole saliva protein constituents, high adhesion capability to albumin and amylase as well as high affinity toward S. sobrinus.

It has been reported that Lactobacilli and Streptococci species create a low pH oral environment (pH < 5.5) due to the bacterial by products[15]. S. mutans is strongly associated with the initiation of dental caries,[16] and Lactobacilli have long been associated with caries development[12]. Therefore, determination of both the S. mutans and the Lactobacilli counts was considered important to determine oral hygiene risk in this study. Also, whether the counts increase over a period of time causing increasing the effects of the colonization was important to be determined.

In this study, bacterial sampling was performed before bonding (T0), one week later, 5weeks later and 8 weeks later. The pre-treatment sampling (T0) was selected to check for presence of any contamination with the elastomeric modules in as received state from the company. T1 (at the end of 1 week) was selected as Sukontipark et al detected abundant plaque on bonded teeth with one week. The third occasion (T2) was performed four weeks after the second one (T1), which was equivalent to the average duration between orthodontic appointments. The fourth occasion (T3) was performed at the end of 8th week to study the colonization over a longer duration of time.

The study showed the absence of contamination of packed elastomeric modules i.e. T0 sample. This is similar to the results obtained by Giovana et al who evaluated in vitro the surface of elastomerics to verify the presence of pathogenic microorganisms at the moment of unpacking and results obtained were, that no microorganism growth was observed at the moment of unpacking.

The result of the current study revealed that the elastomeric rings used for ligation of the wire on the teeth exhibited presence of Streptococcus mutans and Lactobacilli microorganisms and there was a progressive increase in the colonization at different intervals of time.

Fixed orthodontic appliances significantly increase the colonization of Streptococcus mutans and Lactobacilli, as shown in several studies[1,2,17,19].

These results obtained in this study are in accordance with studies done by Forsberg et al[2] who evaluated microbial colonization of patients treated by fixed orthodontic appliances and reported a significant increase in the number of Streptococcus Mutans and Lactobacilli in saliva after 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.

This increase seen in Streptococcus mutans and lactobacilli in this study was also in accordance with studies done by Turkkahraman[2] who determined the changes in the microbial flora and periodontal status after orthodontic bonding and concluded that fixed orthodontic appliances significantly increase Streptococcus mutans and Lactobacilli.

Also, the results of the study by Magno et al. showed that, after a 15-day intraoral period, S. mutans colonies or biofilms were observed on the entire surface of 85% of the elastomeric rings by a ranked scale analysis.

This study shows that, at the end of 1st week, 12 samples out of 30 showed presence of Streptococcus Mutans which was statistically significant. However, only 4 samples out of 30 showed growth of Lactobacilli which was statistically insignificant growth at the end of 1st week.

Though there was presence of lactobacilli in a few samples, this statistically insignificant growth was not in accordance with study by Turkkahraman who showed a statistically significant presence of lactobacilli at the end of 1st week. A feasible explanation may be the differences in sample size, sample teeth, statistical methods or the study design.

The colonization of Streptococcus Mutans was higher than Lactobacilli (graph 1) at all the intervals of time i.e. at the end of 1st, 5th & 8th week and was progressively increasing and it was statistically significant ($P<0.05$) at the all the time intervals i.e. T1, T2 and T3 as well.

The mean value of Streptococcus mutans increased from 0.00 at T0 to 10.6 at T1 followed by which it increased to 50.3 at T2 and reached up to 89.6 at T3. As Streptococcus is strongly associated with the initiation of dental caries, it is postulated that the risk of demineralization is high.

Although, Lactobacilli was seen in just 4 samples out of 30 at T1, with a very low count, that is statistically insignificant, this count increased progressively increased at T2 and T3 with the mean count increasing from 18.6 at T2 to 26.8 at T3. However, this count was less than Streptococcus mutans count at all duration.

This shows that if the elastomeric modules are continued for 2months duration of time, the colonization increases to a significant level, up to almost one and a half times more at T3, than at the T2 period which is usually the duration of appointment interval for orthodontic patients.

Although literature does not mention any specific time period of changing the modules, but based on this study, it is best to change the elastomeric modules at the end of 5th week even if an orthodontist continues the same wire for prolonged periods for the maintenance of patients' oral hygiene.

Thus, on examination of orthodontic attachments over a scheduled period of time microorganisms were isolated which indicates that patients undergoing fixed mechanotherapy and ligated with elastomeric modules should exercise maximum oral hygiene with oral

hygiene adjunctive aids other than routine brushing and should also undergo periodic oral prophylaxis so as to avoid any iatrogenic damage and alteration of the oral environment.

Special care was also taken in the randomization of the collected modules in the oral environment to avoid bias related to different hygiene control individual patterns.

The use of elastomeric chains may introduce broader retention areas for plaque accumulation and the plaque accumulated on the exposed material may have detrimental effects on the surrounding hard and soft tissues, since its proximity to bracket margins may enhance the possibility of undesirable effects such as enamel decalcification or gingival inflammation.[20]

This study evaluated the adherence of two micro-organisms to the elastomeric ligatures namely Streptococcus mutans and Lactobacillus. Periodontal pathogens such as Actinobacillus actinomycetemcomitans and Tannerella forsythia have also been significantly associated with gingival inflammation during orthodontic therapy. As further reference to this study, the microbial spectrum could be identified on the elastomeric modules.

This study estimated the bacterial adhesion only on the respective ligatures. Estimation of altered micro flora in and around the environment of the ligature is a limitation in this study and could be pursued utilizing improved technologies, DNA isolation, and polymerase chain reactions.

Further investigations are needed to provide detailed information about the several commercial brands of conventional and modified elastomeric rings available before they can be advised for clinical use as orthodontic ligation accessories with lesser bacterial biofilm formation.

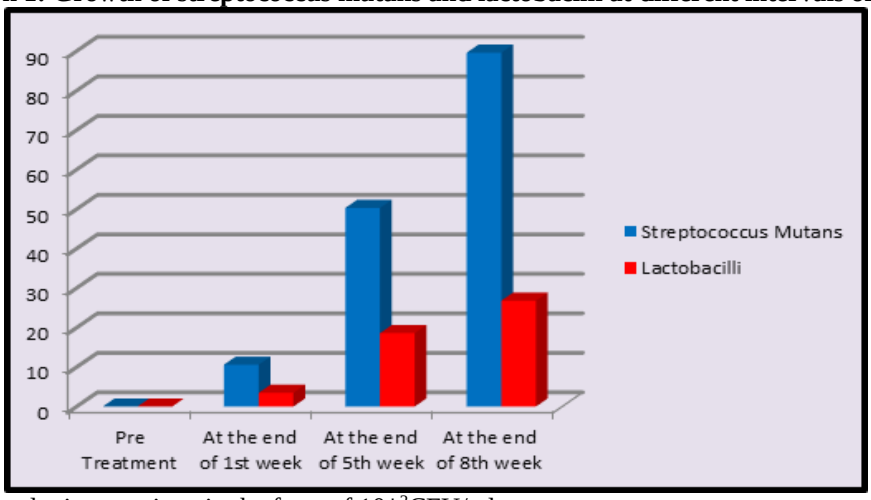
Conclusion

There is significant presence of microbial colonisation of Streptococcus mutans and Lactobacillus on the elastomeric modules during orthodontic treatment. However, presence of Lactobacilli is not significant at the end of 1stweek. On placement of elastomeric modules for ligation and its evaluation at different intervals of time i.e. 1^{st,} 5th and 8th week, there is statistically significant rise in the microbial colonization of Streptococcus mutans & Lactobacilli during the orthodontic treatment. Since these organisms are involved in the initiation and progression of caries, patients undergoing fixed orthodontic treatment and ligated with elastomeric modules should exercise maximum oral hygieneso as to avoid any alteration of the oral environment and subsequent iatrogenic damage.

Table 1: Streptococcus mutans and Lactobacilli at different intervals of time

Sample no.	T0 Pre treatment		T1 At the end of 1st week		T2 At the end of 5th week		T3 At the end of 8th week	
	Streptococcus mutans	Lactobacilli	Streptococcus mutans	Lactobacilli	Streptococcus mutans	Lactobacilli	Streptococcus mutans	Lactobacilli
1	NG	NG	NG	NG	50	15	80	25
2	NG	NG	10	NG	20	10	75	26
3	NG	NG	NG	NG	45	27	120	15
4	NG	NG	60	NG	56	32	140	50
5	NG	NG	NG	3	82	15	90	20
6	NG	NG	NG	NG	70	24	90	30
7	NG	NG	NG	NG	60	20	130	30
8	NG	NG	NG	2	30	14	75	25
9	NG	NG	4	NG	40	8	95	15
10	NG	NG	NG	NG	56	15	100	27
11	NG	NG	15	NG	70	27	98	15
12	NG	NG	NG	NG	80	23	82	38
13	NG	NG	NG	NG	36	18	60	26
14	NG	NG	NG	NG	30	20	70	30
15	NG	NG	28	NG	50	15	85	15
16	NG	NG	NG	NG	70	30	98	40
17	NG	NG	23	NG	56	24	128	45
18	NG	NG	30	7	70	9	102	10
19	NG	NG	34	2	66	30	96	45
20	NG	NG	NG	NG	70	25	118	30
21	NG	NG	NG	NG	50	18	86	26
22	NG	NG	50	NG	60	3	85	20
23	NG	NG	NG	NG	35	10	90	8
24	NG	NG	30	NG	50	15	100	20
25	NG	NG	NG	NG	20	25	60	35
26	NG	NG	NG	NG	30	30	50	40
27	NG	NG	24	NG	58	8	78	17
28	NG	NG	NG	NG	60	16	110	20
29	NG	NG	NG	NG	49	13	69	28
30	NG	NG	10	NG	30	20	60	33

Graph 1: Growth of streptococcus mutans and lactobacilli at different intervals of time



Note: numbers of colonies are given in the form of 10^{*3}CFU/ml.

Graph 2: Progressive colonization of streptococcus mutans and lactobacilli at different intervals of time

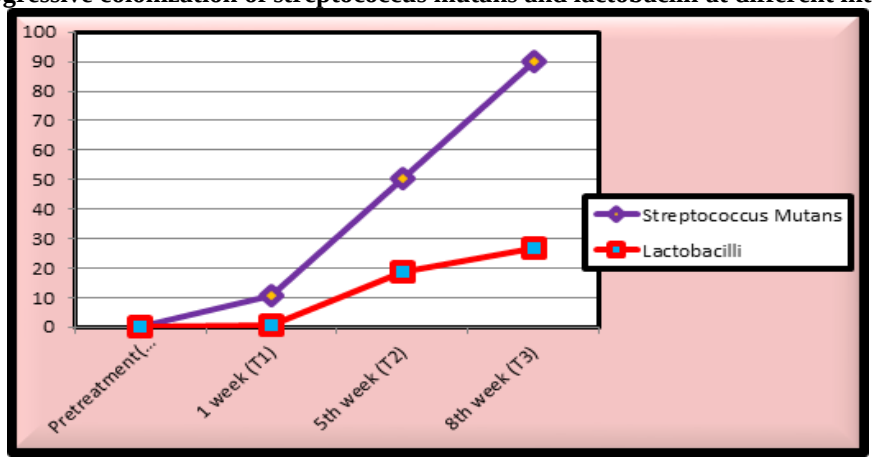


Table 2: Mean, Standard error of mean & Standard Deviation of Streptococcus Mutans & Lactobacilli at different intervals

	Mean		Standard deviation		Standard error of mean	
	S. mutans	**Lactobacilli**	**S. mutans**	**Lactobacilli**	**S. mutans**	**Lactobacilli**
At pretreatment (T0)	0.000	0.000	0.000	0.000	0.000	0.000
At the end of 1st week(T1)	10.6	0.467	16.625	1.432	3.035	0.261
At the end of 5th week(T2)	50.3	18.633	17.217	7.677	3.143	1.402
At the end of 8th week(T3)	89.667	26.800	22.394	10.675	4.088	1.949

Table 3: Comparison of Streptococcus Mutans& Lactobacilli at different intervals

Time Interval	**Streptococcus mutans**		**Lactobacilli**	
	Z value	**P value**	**Z value**	**P value**
Pretreatment to end of 1st week	3.062	P = 0.002*	1.841	P = 0.06
1st to 5th week	4.764	P < 0.001*	4.785	P < 0.001*
5th to 8th week	4.753	P < 0.001*	3.792	P < 0.00016*
1st to 8th week	4.783	P < 0.001*	4.786	P < 0.001*

*Statistically Significant

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