Original Article

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Dentistry Section

Comparison of Contamination of Low-Frictional Elastomeric Rings with That of Conventional Elastomeric Rings by *Streptococcus mutans* - An In-vivo Study

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ABSTRACT

Introduction: The presence of brackets and ligatures has been shown to be related to an increase in gingival inflammation and increased risk of decalcification. The various measures were taken to reduce the plaque accumulation and also lot of efforts were made by manufacturers that reduced the binding friction between the ligature rings and arch wire that facilitated easy sliding of the tooth through the wire. The low frictional ligatures rings manufactured by different manufacturers presumed to attract fewer bacteria due to greater reduction in surface roughness. Our study aimed to evaluate whether the low frictional elastomeric rings accumulate fewer bacteria than conventional ligature rings.

Materials and Methods: Thirty patients (15 males and 15 females) who underwent fixed appliance therapy were selected. The study was done using split-mouth design. In each volunteer, synergy low frictional elastomeric rings were tied to brackets bonded to the maxillary premolar on the right side and mandibular premolar on the left side. Conventional elastomeric rings that served as control group were tied to the contralateral teeth, with the same design. Samples were collected after four weeks (28 days) and cultured for bacteria *Streptococcus mutans*.

Results: There was no statistical difference between *Streptococcus mutans* count in low frictional elastomeric rings with that of conventional rings.

Conclusion: We concluded that adherence of *Streptococcus mutans* is similar in both synergy low frictional elastomeric rings and conventional clear elastomeric rings and thus the manufacturer's claim of minimal bacterial adherence was discarded.

Keywords: Bacteria, Ligature, O rings

INTRODUCTION

Dental caries is one of the most common infectious diseases in the human oral cavity. The enamel and dentin are demineralized by acids, such as lactic acid, which are produced as a by-product of the carbohydrate metabolism by cariogenic bacteria in dental plaque [1]. Among the oral bacteria, *Streptococcus mutans* have been implicated as major cariogenic bacteria [2]. There is increased risk of decalcification and gingival inflammation in orthodontic patients that lead to demineralisation and white spot lesions [3-6].

The various measures taken to reduce the plaque accumulation included use of chlorhexidine or fluoridated mouth washes, bonding using fluoride containing bonding agents, fluoride releasing bracket bases and ligature rings. In the recent times the elastomeric ligature rings had replaced the steel ligatures due to its ease of application that saved clinicians time [7].

Efforts made by orthodontic product manufacturers reduced the binding friction between the ligature rings and arch wire thereby facilitating easy sliding of the tooth through the wire. A material with high Surface Free Energy (SFE) will attract more bacteria to its surface than one with low SFE according to thermodynamic rule [8]. The low frictional ligatures rings manufactured by different manufacturers using their own different technology (super slick by TP orthodontics using metafasix technology, synergy low friction by Rocky Mountain Orthodontics, Alastic rings by 3M) are presumed to attract fewer bacteria due to greater reduction in surface roughness. Our aim was to compare the effect of contamination of *Streptococcus mutans* in low frictional synergy elastomeric rings and conventional clear elastomeric rings.

AIMS AND OBJECTIVES

The aim of this study was to evaluate the amount of *Streptococcus mutans* contamination on synergy low friction elastomeric modules and to compare it with that of clear conventional modules. A null hypothesis that there was no difference in friction between conventional ligature rings and low frictional ligature rings was created.

MATERIALS AND METHODS

The present study was a prospective study carried out in Department of Orthodontics and Dentofacial Orthopaedics, Manipal College of Dental Sciences, Mangalore, Karnataka, India. This study was approved by the institutional Ethical Committee.

Forty five patients who underwent fixed appliance therapy at Manipal College of Dental Sciences, Mangalore were screened. Thirty patients (15 male and 15 female) were selected who took part in the study based on the inclusion criteria. The mean age of population was with a range of 14 y to 26 y.

The patients are selected based on the following inclusion criteria:

- Patients who underwent treatment with pre adjusted edgewise appliance (0.022" x0.025" slot stainless steel brackets with 0.019"x0.025"stainless steel wire) by either extraction or non-extraction treatment protocol.
- Patients were not using any antimicrobial mouthwash during the experimental period.
- Patients had no systemic disease or illness.
- Patients who had not used antibiotics in any form within the

previous three months from the experimental period and /or using them during experimental period.

- Patients whose teeth had brackets that were not bonded with glass ionomer cement and/or any fluoride containing adhesives.

The purpose of the study was explained to the subjects and informed consent was obtained from the patients or their parents (or legal representatives).

Prior to onset of study all subjects were advised thorough oral prophylaxis, to make them free of any plaque and calculus at baseline level.

The study was done using split-mouth design. In each volunteer, Synergy low frictional elastomeric rings (silicone injected, opaque white, reduced friction, stain resistant, low profile, code-J00151, Rocky Mountain Orthodontics) were tied to brackets bonded to the maxillary premolar on the right side of the dental arch and mandibular premolar on the left side, for a total of 60 ligatures. Conventional elastomeric rings (transparent, low profile code-J00345, Rocky Mountain Orthodontics) that served as control group were tied to the contralateral teeth, with the same design and also totalling 60 ligatures [Table/Fig-1-3].

The patients were given regular oral hygiene instructions and advised to brush twice using non-fluoridated tooth paste. However, no dietary restrictions were recommended for the patients. Patients were instructed not to eat, drink or rinse mouth one hour prior to sample collection.

Samples were collected after four weeks (28 days) from the day of placement at 11.00 am to 11.30 am with sterile explorer tip for each sample under sterile environment and were placed in separate sterile test tube [Table/Fig-4]. The samples were transported to The Department of Microbiology, Kasturba Medical College for further processing. All samples were cultured within half an hour.

Collected samples were first removed from the test tube using a Nichrome loop and then dropped in test tube containing 1ml peptone water and mixed vigorously for 60 sec [Table/Fig-5]. 0.01ml of this solution was inoculated on the agar plate that contained the culture medium (Salivarius mitis agar with 20% sucrose and 0.2units/ml bacitracin) and a single streak was made across the centre. Once the primary inoculum was made, the same loop was used to spread the material on the plate. The inoculum was successively streaked with a back and forth motion into each quadrant by turning the plate at 90° angles [Table/Fig-6]. The purpose of this process was to dilute the inoculum sufficiently on the surface of the agar medium so that well isolated colonies of bacteria known as colony forming units can be obtained [9]. The agar plates were incubated for 48 h at 37°C. The number of colonies of *Streptococcus mutans* were counted [Table/Fig-7] and identified by their colonial morphology [9]. Smears were taken & *Streptococcus mutans* were confirmed by gram staining method and microscopic examination.

STATISTICAL ANALYSIS AND RESULTS

Streptococcus mutans count was obtained by dispersing 0.01ml of solution on the agar plate, the resulting colony count was multiplied by 10^2 to obtain the colony forming units/ml.

[Table/Fig-8] shows the mean *Streptococcus mutans* values and the standard deviations in each of the four groups, which were calculated. The mean *Streptococcus mutans* count for synergy low frictional elastomeric rings (7.8 X 10^3 with std.dev of 21.5 X 10^3) was less than that of conventional clear elastomeric rings (9.2 X 10^3 with std.dev of 17.2 X 10^3). But the median value of synergy low frictional elastomeric rings (7.5 X 10^2) was more than that of conventional clear elastomeric rings (3.2 X 10^3). However, the difference in the *Streptococcus mutans* growth offered by the two groups was not statistically significant (p >0.005). Thus, these results showed no clear differences between the *Streptococcus mutans* growth observed on the tested modules.

	N	MEAN	STD.DEV	MEDIAN	Wilcoxon Signed Ranks Test	p-Value
UR & LL	30	7.8X10^3	21.6X10^3	7.5X10^2	1.346	0.178
UL & LR	30	9.2X10^3	17.2X10^3	3.2X10^3		

[Table/Fig-8]: Comparison of average S *mutans* of the study and control group p<0.05 is significant

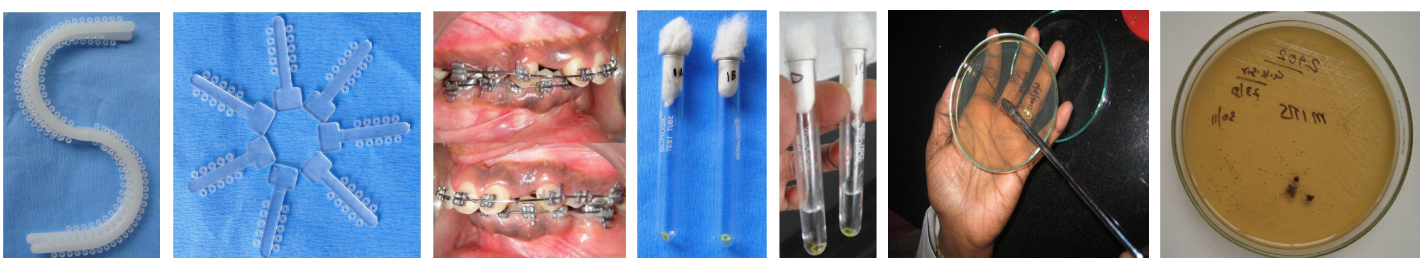
The *Streptococcus mutans* count observed for each sample is shown in [Table/Fig-9]. The results showed that the maximum contamination occurred in conventional clear modules on upper left side (mean = 9.2 X 10^3 ; std.dev of 33.9 X 10^3;median of 10^3) and least contamination was observed in conventional clear modules on lower right side (mean=2.8 X 10^3; std.dev of 25.1 X 10^3; median of 5.5 X 10^2) with synergy low frictional elastomeric rings values in between(mean=7.8 X 10^3; std.dev of 25.1 X 10^3) for both upper right and lower left. However, the results were statistically non-significant (p>0.05).

	N	Mean	STD.DEV	Median	Wilcoxon Signed Ranks Test	D.F	p-value
UR	30	7.8X10^3	25.1 X 10^3	8 X 10^2	2.371	3	0.499
LL	30	7.8X10^3	25.1 X 10^3	10^2			
UL	30	9.2X10^3	33.9 X 10^3	10^3			
LR	30	2.8 X 10^3	25.1 X 10^3	5.5 X 10^2			

[Table/Fig-9]: *Streptococcus mutans* count observed for each sample p<0.05 is significant

The data was further analysed using pairwise comparison through Wilcoxon signed ranked test. [Table/Fig-10] shows the comparison between the four groups using the Wilcoxon signed ranked test. The values of Wilcoxon rank test and the corresponding values of 'p' were tabulated in [Table/Fig-10]. These comparisons (p>0.005) showed that no significant difference existed in the performance of the four types of the elastomeric modules tested, i.e. the *Streptococcus mutans* growth found on synergy low frictional elastomeric modules was almost same as that found on the conventional clear modules. Also, there was no significant difference found between *Streptococcus mutans* contamination when the upper and lower arch and right and left sides were compared (p>0.008).

There was no significant difference found between *Streptococcus mutans* contamination on comparing the upper and lower arch and also right and left sides (p>0.008).



[Table/Fig-1]: Synergy low frictional elastomeric rings **[Table/Fig-2]:** Conventional elastomeric rings **[Table/Fig-3]:** Samples placed in premolars using split mouth design **[Table/Fig-4]:** Collection of samples in sterile test tube **[Table/Fig-5]:** Test tube containing 1ml peptone water **[Table/Fig-6]:** Inoculating sample solution into culture medium **[Table/Fig-7]:** Colony forming units of *Streptococcus mutans*

		Mean Difference	Wilcoxon Signed Ranks Test	p-value
Upper Right	Lower Left	-10^3	0.45	0.654
	Upper Left	$-7.8X10^3$	1.53	0.127
	Lower Right	$-5X10^3$	0.24	0.808
Lower Left	Upper Left	$-7.8X10^3$	1.46	0.144
	Lower Right	$-5X10^3$	0.58	0.562
Upper Left	Lower Right	$-12.7X10^3$	1.51	0.130

[Table/Fig-10]: Pairwise comparison between the four groups
p<0.008 is significant

[Table/Fig-11] summarises the mean *Streptococcus mutans* count between male and female in each group analysed by Wilcoxon signed rank test and shows that there was no significant difference found between *Streptococcus mutans* count in male and females in each group (p>0.05).

	Sex	N	Mean	STD.DEV	Median	Wilcoxon Signed Ranks Test	p-value
A	Female	15	$14.7X10^3$	$34.7X10^3$	10^3	1.53	0.125
	Male	15	$7.5X10^2$	$8.1X10^2$	10^2		
	Total	30	$7.8X10^3$	$25.1X10^3$	$8X10^2$		
B	Female	15	$8X10^3$	$25.6X10^3$	10^3	1.33	0.183
	Male	15	$7.6X10^3$	$25.7X10^3$	10^2		
	Total	30	$7.8X10^3$	$25.2X10^3$	10^2		
C	Female	15	$8.7X10^3$	$25.5X10^3$	10^2	1.22	0.224
	Male	15	$22.4X10^3$	$40.3X10^3$	$1.3X10^3$		
	Total	30	$15.5X10^3$	$33.9X10^3$	10^3		
D	Female	15	$3.7X10^3$	$4.7X10^3$	10^2	0.42	0.671
	Male	15	$2X10^3$	$3.3X10^3$	10^3		
	Total	30	$2.8X10^3$	$4.1X10^3$	$5.5X10^3$		

[Table/Fig-11]: Comparison of *S.mutans* in male and female in each group

DISCUSSION

Both periodontal disease and dental caries are multifactorial. The plaque and the bacteria present in the oral cavity contribute to the initiation and progression of periodontal disease and dental caries. However, when situations arise creating 'ecological stress', there is a shift in the microbiological balance, leading to appearance of cariogenic and/or periodontopathic bacteria [10]. Among the bacteria present in this flora, *Streptococcus mutans* is the prime organism responsible for dental caries activity initiation [2].

Bloom et al., showed a definite increase in *Streptococcus mutans* and lactobacilli count which was strongly associated with orthodontic appliances [11]. Balansifen et al., showed a significant drop in plaque pH and increased bacterial count was related to orthodontic materials [12].

Bacterial accumulation on dental material was determined by various surface characteristics. The adhesion of bacteria was significantly affected by high surface roughness values because of a reduction of shear forces on initially attaching bacteria. Surface free energy values of material are directly proportional to adhesion of bacteria to that material. The chemical composition of the material, surface hydrophobicity, and the zeta potential also affect bacterial adhesion [3].

Till date many researchers have done various studies to study the influence of various orthodontic materials on accumulation of *Streptococcus mutans* count and also its role inenamel demineralisation. Quirynen et al., explained that the surface roughness of the substrate material was more important than the surface free energy of the material in facilitating bacterial adhesion [13]. Forsberg et al., reported that the archwire ligated with an steel wire exhibited a lesser number of microorganisms than the teeth ligated with elastomeric ring. The insertion of fixed appliances increased the level of lactobacilli and *Streptococcus mutans* in saliva. Forsberg et al., also proved that the accumulation of plaque is more in elastomeric rings compared to stainless steel ligatures [14]. This may be due to the combination of plastic and filler that might provide rough surfaces which will increase the adhesion of cariogenic streptococci [15]. Quirynen et al., explained that the surface roughness of the substrate material was more important than the surface free energy of the material in facilitating bacterial adhesion [13].

Scanning electron microscopy study by Sukontapatipark W et al., concluded that there was no correlation between method of ligation and the bacterial adhesion on either enamel surfaces or composite [16]. However, the archwire was not ligated into the bracket because the experimental design included only one bonded tooth in each quadrant. Since, the posterior teeth accumulate more plaque, our study used the microbial samples collected from upper second premolars. In addition, not in all cases, the anterior teeth bonding was done in initial stages of the treatment.

Equal numbers of male and female patients were studied to avoid any bias in sex as earlier literatures showed evidence of increased caries potential in females compared to males which in turn means increased *Streptococcus mutans* in females [3]. However, our study showed no statistically significant difference between the two groups. These results correlated with that of Zichert [17] and Ogaard [4] who in their study found that there was no difference in the caries activity between male and female patients.

Age group in the range of 14 to 26 y with mean age of 19.3 y were selected as most of the patients who sought orthodontic treatment fell into this category. Also, almost all teeth till second molars were erupted in these patients. The patients with active carious lesions were also excluded from the study to eliminate the bias as *Streptococcus mutans* count was positively correlated with active carious lesion [1-3].

Because the primary aim of the study was to evaluate the importance of elastomeric rings on the recolonization pattern of mutans streptococci, a split mouth design was used, with each subject being his/her own control. Carrying out experimental procedure in this way had one more advantage that allowed to keep the number of participating subjects relatively low.

Ferreira Magno et al., [8] did an in-vivo evaluation and concluded that Super Slick elastomeric rings had significantly greater contamination by *S mutans* than conventional elastomeric rings and there was no clinical evidence that Super Slick elastomeric rings were effective in reducing bacterial biofilm formation on their surfaces, and a recommendation for their use in orthodontic therapy for that purpose is not justifiable.

However, the scanning electron microscopy (SEM) used in his technique does not carry much advantage compared to our study design as SEM technique too lacks the ability to identify species [17] and also it did not analyse the bacteria clinging to all sides of the elastomeric rings. Our study had the advantage that accounted all the dispersed bacteria into the solution for calculation and also was cost effective when compared to SEM study.

The method of bacterial culture used in our study was the most traditional method of *Streptococcus mutans* culture that used Mitis salivarius agar with 0.2%/ml bacitracin and 20% sucrose. This method was the most frequently used medium for isolation of mutans streptococci, probably because it had the ability to distinguish and select for this microorganism when used with conventional dilution and plating techniques [18].

The values obtained from our study were highly skewed and the bacterial counts were in wide range (minimum of 102 to maximum of 105). This wide variation occured due to difference in the bacterial growth, which was multifactorial. Several factors such as age, dietary habits, salivary flow, salivary pH, viscosity, buffering capacity

of saliva, socio economic status, oral hygiene measures and hereditary factors play an important role in growth and adhesion of *Streptococcus mutans* [19].

The wide range of bacterial count was also observed in different zones in the same patients. This difference was quite confusing, but the probable explanation might be due to the difference in brushing habits and also the difference in thickness of adhesive surrounding the bracket. The difference in adhesive thickness might be due to clinicians' variability in removing the excess adhesive around the bracket. This excess adhesive around the bracket base was an obvious predisposing factor for plaque accumulation due to the increase in surface roughness provided by it [20].

The results obtained from our study showed that there was no difference in bacterial accumulation found on low frictional elastomeric rings with that of conventional elastomeric rings and our findings correlated with that obtained through SEM study by Ferriero Magno et al., [8]. However, our study differed from his study by material of choice, its difference in manufacturing procedure (Metafasix technology used in Super slick elastomers against silicone injection technique in our sample) and also method of analysing bacteria (SEM study against traditional bacterial culture).

Magno et al., assessed the surface characteristics of Superslick and non-Superslick conventional elastomeric ligatures and concluded that the loss of polymer layer acts as a niche for greater plaque accumulation [8]. Griffith et al., also noticed a higher frictional resistance offered by Superslick ligatures which may be also due to probable loss of polymer coating during placement of the elastomeric ring around bracket [21].

The surface property of the material can be changed during the fabrication procedure, which can increase the surface free energy, because the physical and chemical changes of materials can significantly affect the relevant physicochemical surface properties [22]. This necessitates further studies to analyse the surface characteristics of the materials used in our study.

Our study concluded that there was no significant difference found between the Synergy low frictional elastomeric rings and the conventional clear elastomeric rings and the null hypothesis was accepted. Further studies on this topic are recommended with a study design made using advanced microbiological identification techniques.

CONCLUSION

The results of the present study revealed that there was no significant difference between the Synergy low frictional elastomeric rings and conventional clear elastomeric rings.

Thus, we concluded that adherence of *Streptococcus mutans* is similar in both Synergy low frictional elastomeric rings and conventional clear elastomeric rings and thus the manufacturer's claim of minimal bacterial adherence can be discarded.

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