

The Effect of Fluoride-releasing Elastomeric Chains on *Streptococcus mutans* Levels in Saliva and Dental Plaque in Orthodontic Patients

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Abstract

Background and Aim: During fixed orthodontic treatments the absolute number and percentage of salivary *Streptococcus mutans* increase. This will cause increase of enamel demineralization and dental caries. The purpose of present study is to evaluate the effect of fluoride-releasing elastomeric chains in the *streptococcus mutans* levels in saliva and bacterial plaque.

Materials and Methods: In this randomized clinical trial sixty patients, who were under fixed-orthodontic appliance treatments were selected and randomly divided into two groups of 30 each. Conventional elastomeric chains were used in group 1. As for the second group fluoride-releasing elastomeric chains were utilized. Four samples of saliva collected on days 0, 7, 14 and 28, and two plaque samples were collected on the beginning and finishing days in both groups. The fluoride-releasing and conventional elastomeric chains were removed on day 28. All samples were then used for microbial culture to count the *Streptococcus mutans* colonies. The results were analyzed statistically with the repeated measures Analysis of Variance (ANOVA) and Student t-tests.

Results: The result of *Streptococcus mutans* (CFU) in saliva at each time point in the study showed no statistically significant difference between the two groups ($p \geq 0.301$). Comparison of the effect of elastomeric chains on the *Streptococcus mutans* colonization in dental plaque and elastomeric chains surface, also did not show any statistically significant difference between the two groups ($p = 0.317$ and 0.803 respectively).

Conclusion: There was no clinical evidence that fluoride-releasing elastomeric chains were effective in reducing the formation and colonization of *Streptococcus mutans* in saliva and dental plaque.

Key Words: Streptococcus mutans, Elastomeric chain, Fluoride, Caries

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Introduction

During fixed orthodontic treatment the absolute number and percentage of *Streptococcus mutans* increase in saliva and dental plaque [1-4]. This will

cause increased enamel demineralization and dental caries [5-7]. Increase of white spot lesions have been reported in 50% of patients. As caries may appear clinically around brackets even after one

month during orthodontic treatment, the use of appropriate method to inhibit demineralization of the enamel is important [8]. Preventive programs, including oral hygiene instructions, fluoride tooth pastes and fluoride mouth washes are likely to be more effective at reducing dental caries. However an ideal preventive program should not be dependent on patient cooperation [9]. Attempts for reducing and prevention of dental caries have been led to introducing many fluoridated products such as glass ionomer cements and modified resin glass ionomers, [10-12]

The use of fluoridated adhesives in order to reduce cariogenic biofilms has been also recommended.¹³ The effect of fluoridated orthodontic adhesives with or without fluoridated mouth washes have been also evaluated and their positive accumulative effects have been demonstrated [14-15].

One of the factors which cause accumulation of microorganisms during orthodontic treatment is the use of elastomeric materials. On the other hand, elastomeric materials (modulus and chains) easily accumulate microorganisms and bacterial plaques during orthodontic treatments. This is the reason why manufacturers have focused to present materials which reduce bacterial population. Several studies have focused on fluoride release from these materials. Storie et al. have evaluated mechanical properties and fluoride releasing capacity of stannous fluoride from fluoride releasing elastomeric chains [16].

It has been demonstrated that concentration of released stannous fluoride from this kind of elastomeric ties was high at first but its level decreases after one week [9].

Also it has been shown that use of such materials temporarily decreases the number of salivary *S.mutans* and increases the resistance of enamel up to a depth of 20 μ m after one month of try-in-mouth.⁹ Due to the longer span of fluoridated elastomeric chains than elastomeric ties, it is supposed that they may have more such effect on population of *S.mutans*. Moreover Based upon this hypothesis the objective of the present study was to evaluate the efficacy of fluoride-releasing elastomeric

chains in the control of *S.mutans* levels in saliva and plaque surrounding the orthodontic appliances.

Methods and Materials

This study was a prospective, randomized clinical trial study, which was approved by the Ethical Committee of Shiraz Medical Science University (process # 2011). A written informed consent was obtained from the participating patients. This study had a parallel group design, in which one group received the experimental material and the other considered as a control. Based on a 1:1 ratio between groups, a sample size of 60 patients would give more than 80% power to detected significant differences with a 0.35 effect size and at $\alpha = 0.05$ significance level.

Participants included sixty patients with the age range of 18 to 28 years (mean 21 ± 0.46 years), who were at the beginning stage of fixed-orthodontic treatment. They were recruited from patients in the orthodontic department. Patients using any kind of antibiotics within the previous 2 weeks, antimicrobial mouthwash, or having any systemic disease were excluded from the study. The patients were randomly divided into two groups of thirty. Fluoride-releasing elastomeric chains (Fluor-I- Chain, Ortho Arch Co. Inc., USA) were applied to the experimental group and conventional elastomeric chains (Ortho Technology, Tampa, Florida USA) were used in the control group. The randomization was accomplished using random numbers in a block design. Elastomeric chains had the same size and were closed loop type chains in grey color for both groups. Standard oral hygiene instructions consisted of guiding patients through brushing their teeth with sodium fluoride toothpaste (Crest, Procter and Gamble, Cincinnati, Ohio) and orthodontic brush (Oral B-15, Cooper Care, Palo Alto, Calif.) accompanied by flossing. Before starting the procedure, all teeth involved in the study were evaluated clinically and teeth with abnormal size and shape or any restoration on buccal surfaces were excluded. One sample of non-stimulated whole saliva was collected at the beginning of the study (day 0) from both groups to determine the

number of *streptococcus mutans* CFUs. Plaque samples were also collected from the area surrounding the bonded bracket of upper right second premolar with a sterilized curette (EXSB, HU-Friedy, Leimen, Germany). After transferring the samples, patients received 3 loops of elastomeric chains in both groups (fluoridated type for the study group and conventional nonfluoridated type for the control group) which were tied from upper right canine to upper right first molar. On days 7, 14, 28, saliva was collected from both groups, while plaque samples were collected on day 28. The used elastomeric chains were aseptically removed after 28 days using sterilized Mathieu pliers (Dentaurum, Pforzheim, Germany), placed in separate containers. All samples were taken to the laboratory within 10 to 15 minutes. To assure the absence of *S.mutans* contamination during manufacturing and package, additional 3 loops of fluoridated and conventional elastomeric chains were removed from their original package and submitted for microbiological culture. All of the plaque and elastomeric chain samples were transferred to sterilized microtubes containing 1 ml of distilled water, while saliva samples were transferred to sterilized microtubes. Samples were homogenized for dispersion and submitted to tenfold serial dilution. Then 50µL of each dilution was plated equidistantly on tryptone soya-yeast agar with 20% sucrose and 0.2 U/ml bacitracin (TYCSB agar; Sigma, St. Louis, MO, USA) and incubated in a candle jar at 37°C for one to two days.

Data were analyzed using SPSS software for windows (version 11.0 Inc.Chicago. IL, USA) at 5% significance level. The results of the number of salivary *S. mutans* at four time points were analyzed statistically with the repeated measures analysis of variance (ANOVA). Student t-test was used to compare the *S.mutans* CFU changes of plaque and elastomeric chain surfaces between groups.

Results

Thirty patients were recruited in each group of study and control. From the study group only 26

completed the study and of the 30 patients of control group, 27 completed the study.

The result of *S.mutans* CFUs in saliva at each time point of the study showed no statistically significant difference between the two groups ($p=0.301$) (Table 1).

Table 1. The mean and SD of Streptococcus mutans CFUs attached to the elastomeric chains in the test and control groups

Group	Mean	SD	Minimum	Maximum	P.V
Control	113/8	1/6	100	794/3	0/803
Test	120/5	2/1	100	1995/2	

Comparison of the effect of elastomeric chains on the *S.mutans* colonization in dental plaque, did not show any statistically significant difference between the two groups ($p=0.0317$) (Table 2 and graph 1).

Comparison of the number of CFU on elastomeric chain surfaces between two groups showed no significant difference between nonfluoridated and fluoride-releasing elastomeric chains ($p=0.803$) (Table 3).

Discussion

Side effects of orthodontic treatments appeared with time. White spot lesions (WSLs) are among the significant clinical problems after orthodontic appliance removal [2]. WSL is considered as a precursor of frank enamel caries. Introducing many fluoridated products such as glass ionomer and resin modified glass ionomer cements are among the attempts for of caries prevention. Currently elastomeric chains that leach stannous fluoride are available in orthodontics. Fluoride releases from these elastomers close to the sites that are susceptible to demineralization.

S.mutans can be found usually in normal flora of the mouth in healthy status. Therefore presence of this microorganism is not necessarily a sign of caries but increase of their population in enamel surfaces may be the causative for caries development [17].

In this clinical trial, parallel group design was used. Although, topically application of fluoride has been reported to have mostly local effects, but

a slight crossover of fluoride could occur via saliva. In this situation, parallel group design was suggested to be the most appropriate [18-19].

Table 2. Mean and SD of Streptococcus mutans CFUs present in saliva in test and control groups based on the time of sampling

0	Control	153/7	49/3	0	301995/7	0/622
	Test	281/2	32/6	0	301995/1	
7	Control	1220/1	13/8	100	120226/4	0/561
	Test	2110	14/9	100	426579/5	
14	Control	591/2	20/6	100	251188/1	0/980
	Test	160	3/8	100	251188/1	
28	Control	843/9	16/7	100	301995/1	0/587
	Test	1528/6	23	100	301995/1	

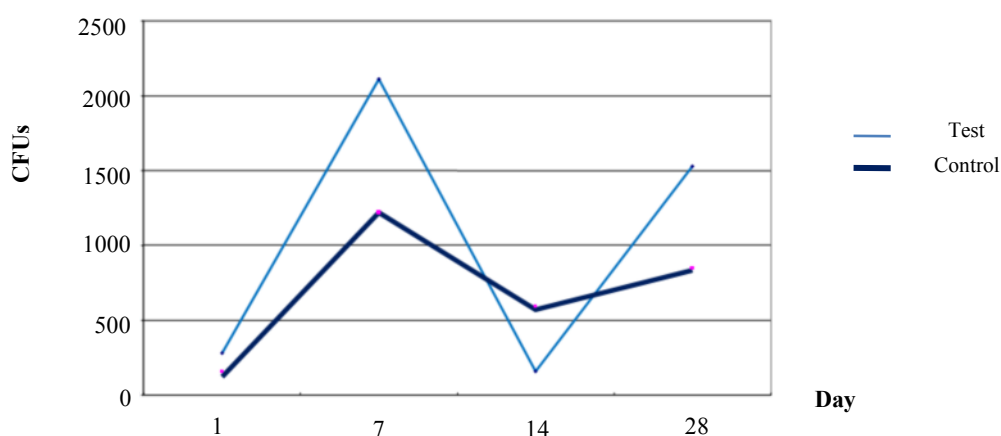


Diagram 1. Change in mean Streptococcus mutans CFUs present in saliva in the two understudy groups at 1, 7, 14 and 28 days

Table 3. Mean and SD of Streptococcus mutans CFUs present in dental plaque in test and control groups based on the time of sampling

0	Control	11/3	16/7	0	15135/6	0/061
	Test	60/3	12/6	0	3019/9	
28	Control	113/8	4/1	100	3019951/7	0/8
	Test	160/4	3/4	100	3981	

The reason for choosing the seventh day of study was based upon the results of some studies which revealed that concentration of released stannous

fluoride was highest on the 7th day and decreased afterwards [9, 20]. The interval of orthodontic appointments is usually 28 days and that was why the

28th day was considered as the final time point for the study.

Microbial culture of elastomeric chains removed from their original package, showed no evidence of *S. mutans* colonies. This is consistent with results obtained from Casaccia et al who found that packed elastomeric chains had no microbial or fungal contamination [2].

In our study, comparison of elastomeric chains contamination in mouth by *S. mutans* did not reveal any difference between the experimental and control groups. This result supports some in vivo studies which claimed no significant difference between fluoridated and conventional elastomeric chains regarding microbial contamination [20, 21]. Although the real reason is unknown, this can be attributed to higher levels of bacterial retention on the elastomeric materials [21]. Garcez et al found that higher biofilm retention is seen on brackets ligated with elastomeric rings compared to those ligated with steel ligature wires and self-ligating brackets [23].

Physical properties of these elastics can be considered as another reason for the lack of significant difference between two groups which caused earlier loss of their elasticity [22]. Wiltshire found that after 1 month in mouth, fluoridated elastomers doubled their weight, but the weight of the non-fluoridated elastomers remained unchanged [24].

This phenomenon might lead to higher bacterial load in fluoridated elastomers. Increase of bacterial colonies on these elastics in comparison to dental plaque may be due to surface roughness of these elastics [2].

Comparison of the number of salivary *S. mutans* colonies or biofilm showed no differences between the two groups. Benson [21] and Mura et al [9] investigated the performance of fluoride-impregnated elastomers on decreasing the number of *S. mutans* in saliva. Their findings indicated that fluoride-releasing elastomeric rings were not effective for such a purpose.

Our study has shown that, after 14 days in mouth, there was a reduction in the *S. mutans* population counts in saliva obtained from both fluoridated and

conventional elastomeric chains. Compared with conventional elastomers, the level of *S. mutans* in saliva were lower with fluoride-releasing elastomeric chains in the second week. However, *S. mutans* count then rose in the fourth week. Results of a study conducted by Wilson & Gregory [25] showed that after placement of the fluoridated elastomers, the percentage of formation of *S. mutans* colonies in saliva decreased significantly, but after 2 or more weeks there was no significant effect. Also Miura et al. found that the number of *S. mutans* in saliva was reduced in the first week with fluoridated elastomeric chains. This reduction however was not significant [16].

The pattern of fluoride release are characterized by an initial burst of fluoride release during the first 48 hours, followed by a diminished release over time [20]. This diminished dose of fluoride possibly does not have any effect on *S. mutans* and leads to lack of any significant differences between the two groups [26].

In our clinical study, there were no statistically significant differences in the number of *S. mutans* in dental plaque in both groups. This confirms the results of the works carried out previously by Mura et al [9] and Benson [21], who suggested that the fluoridated modules do not decrease the number of *S. mutans* colonies in dental plaque. Mota et al [17] and Vande Hoven & Franken [26] found that there was no significant change in the number of *S. mutans* in plaque after application of stannous fluoride. However, production of lactic acid was lower in fluoridated group [17, 26].

Bank et al evaluated the influence of fluoride-releasing elastomers on the development of white spot lesions during orthodontic treatment and found a significant difference in the incidence of demineralized lesions between the experimental and control groups [28]. Fluoridated elastomeric chains and modules have been effective in protection of enamel during orthodontic treatment and reduced decalcification. The strongest effect of these products was observed to be in gingival areas of teeth which were more susceptible to caries [28].

Some studies stated that fluoride concentrations of

less than 0.05 ppm might be effective in reduction of caries. Whiltshire showed that fluoridated elastomeric ties released 1.43 ppm of fluoride following one-month placement in the mouth and then placing them in distilled water for 24 hours which in turn could be influential in caries reduction in susceptible areas [24]. It has been accepted that bactericidal effects against *S. mutans* would be produced from too much higher levels of fluoride than its level in the mouth [22]. This may be the reason for lack of difference between two groups in our study.

The point is that fluoride not only acts on *S. mutans*, but also can effect on enamel to increase its resistant against caries [21]. Fluoride has a high tendency for demineralized areas and therefore can inhibit caries [15].

Although it has not been shown that fluoridated elastomers can reduce population of *S. mutans* in saliva and plaque, they might help reduce the prevalence and severity of white spot lesions during orthodontic treatment by elevating the concentration of fluoride in the plaque adjacent to bracket. Further studies in this realm is highly required.

Another point is that releasing rate of fluoride from these products is high at first and leads to formation of calcium fluoride. Then fluoride leakage will reduce and leads to formation of fluoroapatite which is much more effective in caries protection [24].

Therefore, reduction of fluoride concentration does not mean reduction of its cariogenic protection property.

As a result, to obtain maximum protection, fluoridated elastomers should be used as a part of a preventive remedies for oral hygiene, including fluoride toothpastes and fluoride mouthwashes that are likely more effective at reducing dental caries.

Conclusions

1. There was no difference in the contamination of fluoride-release and conventional elastomeric chains by *S. mutans*.
2. There was no clinical evidence that fluoride-release elastomeric chains are effective in reducing

the formation and colonization of *S. mutans* on saliva and dental plaque. Recommendation for their use in orthodontic therapy for that purpose is not justifiable.

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