**European Journal of Molecular & Clinical Medicine**
**ISSN 2515-8260 Volume 08, Issue 1, 2021**

# Effect of chlorhexidine-releasing elastomeric ligatures on Streptococcus mutans count in orthodontic patients: A clinical trial

**Somayeh Mehrabi [1], Masood Feizbakhsh [1]*, Arezou Tahmorespour [2]**

1. **Somayeh Mehrabi [1]**
Department of Orthodontics, School of Dentistry, Islamic Azad University, Isfahan (Khorasgan) Branch, Isfahan, Iran.
2. **Masood Feizbakhsh [1]***
Department of Orthodontics, School of Dentistry, Islamic Azad University, Isfahan (Khorasgan) Branch, Isfahan, Iran.
**Email:** dr.masood.feizbakhsh @ gmail.com
3. **Arezou Tahmorespour [2]**
Department of Basic Medical Science, Islamic Azad University, Isfahan (Khorasgan) Branch, Isfahan, Iran.

## Abstract

***Objectives***

This study aimed to assess the effect of using chlorhexidine (CHX)-releasing elastomeric ligatures on the number of Streptococcus mutans (S. mutans) in the saliva and dental plaque and on elastomeric ligatures in orthodontic patients.

***Materials and Methods:***

This double-blind clinical trial evaluated 30 orthodontic patients between 14 to 25 years, who were randomly divided into two groups. CHX-releasing elastomeric ligatures were used in the test group while the conventional elastomeric ligatures were used for the control group. Saliva samples were collected at baseline, and 7, 14, 21 and 28 days after the intervention. Plaque samples were also collected at baseline and after 28 days. Elastomeric ligatures were collected in both groups after 28 days. The collected samples were cultured to count the number of S. mutans. Data were analyzed using the Mann-Whitney and Wilcoxon tests.

***Results:***

The salivary count of S. mutans was significantly lower in the test group at 7, 14, 21 and 28 days ($P<0.05$). The S. mutans count on the surface of CHX-releasing ligatures was significantly lower than that on the surface of conventional ligatures ($P<0.05$). However, no significant difference was noted in S. mutans count in plaque samples between the two groups ($P>0.05$). The salivary level of S. mutans significantly decreased during the course of study in the test group.

***Conclusion:***

CHX-releasing elastomeric ligatures can significantly decrease the salivary count of S. mutans in orthodontic patients.

**Keywords:** Streptococcus mutans; Chlorhexidine-releasing elastomeric ligatures; Saliva; Dental Plaque.

## Introduction

Oral hygiene maintenance is difficult for orthodontic patients due to excessive plaque retention around orthodontic wires and brackets [1]. This often increases the risk of enamel demineralization and dental caries [2,3]. On the other hand, the speed of demineralization that occurs around fixed orthodontic appliances is higher than the usual speed of development of carious lesions, such that the

first clinical manifestations of enamel demineralization often appear within the first 6 months following the initiation of orthodontic treatment [4]. Al Maaitah et al. [5] reported the prevalence of white spot lesions (WSLs) to be 7% to 71% in 230 orthodontic patients.

Long-term fixed orthodontic treatment lasting for 1 or 2 years would cause some changes in the oral microflora [6]. It can increase the proliferation of cariogenic bacteria, cause periodontal irritation and lead to formation of microbial biofilm on the surface of teeth and orthodontic appliances [7]. Streptococcus mutans (S. mutans) is among the most important bacteria involved in caries development. Its mechanism of action is based on the production of organic acids and dissolution of the mineral content of the enamel [8].

Research is ongoing to find methods to prevent or minimize enamel demineralization during the course of orthodontic treatment. Several methods have been suggested to decrease the risk of enamel demineralization during orthodontic treatment. Some chemicals have also been proposed to enhance the efficacy of mechanical methods to minimize the microbial plaque [9].

Evidence shows that setting weekly reminders for the parents of orthodontic patients would improve their gingival health status in short-term [10,11]. However, its efficacy for reduction of WSLs has not been confirmed [12]. A noteworthy issue is that an ideal preventive program should not rely or depend on patient cooperation [13].

In the recent years, many products were introduced to the market that are capable of releasing antibacterial agents (such as bonding agents releasing fluoride). All these attempts aim to prevent or decrease dental caries. However, there is still no evidence to support the claim that fluoride added to bonding agents can prevent demineralization [14,15]. The results of addition of fluoride to composite resins have also been generally discouraging [16-18]. Since orthodontic elastomeric ligatures are a common site of microbial plaque formation, addition of antibacterial agents to elastomeric ligatures seems logical. By doing so, the antibacterial agent is available where it is most needed. Moreover, elastomeric ligatures are continuously replaced and can serve as a fresh source of antibacterial agent. The primary results of adding antibacterial agents to elastomeric ligatures were promising [19], although addition of fluoride changed the physical properties of elastics and resulted in their faster degradation in the oral cavity [5].

Chlorhexidine (CHX) is a cationic biguanide with broad-spectrum antibacterial activity. It is considered as the gold-standard antibacterial agent due to its high potential to inhibit microbial plaque formation [20]. It has both bactericidal and bacteriostatic activities, and is effective against Gram-positive and Gram-negative microorganisms, fungi and viruses. In low concentrations, CHX binds to the bacterial cell membrane and increases its permeability, resulting in leakage of intracellular components including sodium. In high concentrations, it leads to deposition of bacterial cytoplasm and cell death [21]. Products containing CHX are available in the form of mouthwashes, gels, sprays, toothpastes, lozenges, varnishes and sustained-release drug delivery systems (DDSs). The most important property of CHX is its inherent sustained antimicrobial effect over long periods of time, which is due to the binding of CHX to hydroxyapatites present in the enamel and proteins and glycoproteins present in the saliva and dental plaque, which would result in sustained release of CHX within 12 to 24 hours [22]. However, it should be noted that the saliva flow decreases the sustained antimicrobial activity of products. However, long presence of antibacterial agents in the oral cavity would prevent dental caries during the course of orthodontic treatment [23]. CHX can prevent dental biofilm formation and decrease the salivary level of Streptococcus mutans (S. mutans), which is the main culprit responsible for development of dental caries [24]. Recently, CHX controlled release systems were introduced to further benefit from its antimicrobial properties [25,26]. In this regard, CHX DDSs were introduced to overcome the clinical complications associated with the conventional use of CHX to decrease the salivary level of S. mutans. A previous study used CHX-releasing orthodontic elastomeric ligatures for this purpose [27]. To achieve this goal, one method is to immerse the elastomeric ligatures in CHX. By doing so, a thick coating of CHX is formed on the surface of elastomeric ligatures. However, preservation of this coating and enabling the sustained release of CHX are among the difficulties encountered in use of this technique [28]. Considering the extensive use of elastomeric ligatures in orthodontic treatment, another DDS based on layer-by-layer technique was introduced, which showed successful results in vitro [28].

However, its efficacy has not been tested in the clinical setting on orthodontic patients. Thus, this study aimed to assess the effect of using CHX-releasing elastomeric ligatures on the number of S. mutans in the saliva and dental plaque and on elastomeric ligatures in orthodontic patients.

**Materials and Methods**

In this double-blind clinical trial, sample size was calculated to be 15 in each group considering the minimum difference of 30% between the two groups, 80% study power and 95% confidence interval. The study was approved by the ethics committee of School of Dentistry, Islamic Azad University, Khorasgan Branch (IR.IAU,KHUSE.REC.1397.121) and registered in the Iranian Registry of Clinical Trials (IRCT20181203041837N1).

A total of 30 patients between 14 to 25 years were selected among those presenting to the Orthodontics Department of School of Dentistry, Islamic Azad University, Isfahan branch who required fixed orthodontic treatment. The patients were selected using convenience sampling. All patients and/or their parents/legal guardians signed informed consent forms prior to participation in the study, and then received oral hygiene instructions. They were requested to brush their teeth using Oral-B orthodontic toothbrush and Oral-B interdental brush twice a day. They were also asked to floss their teeth using Oral-B Super Floss. All patients used Crest toothpaste containing 0.3% sodium fluoride during the study period. The patients were requested to refrain from eating and drinking for 2 h prior to sampling. They were also requested not to use mouthwash during the study period.

The inclusion criteria were (I) presence of all natural teeth, (II) requiring fixed orthodontic treatment, (III) no smoking, (IV) absence of extensive dental restorations or fixed partial dentures, (V) no antibiotic use during the study period and the 2 months prior to the onset of study, (VI) absence of periodontal disease prior to treatment onset, (VII) high motivation for oral hygiene maintenance, (VIII) no use of mouthwash during the study period, (IX) no restoration on the buccal surface of the teeth and (X) normal anatomical shape and height of teeth.

The exclusion criteria were (I) systemic disease or medication intake, (II) history of previous orthodontic treatment, (III) history of CHX use for 1 week or longer in the past 2 months, (IV) presence of active carious lesions, and (V) low saliva flow.

The selected 30 patients were randomly divided into test and control groups (n=15).

**Preparation of coating solution:**

CHX diacetate (Sigma Aldrich, St. Louis, MO, USA) was used as antibacterial agent and ethyl cellulose (N100; Sigma Aldrich, St. Louis, MO, USA) was used as polymer to prepare the coating solution [28]. The polymer volume was 2% of the solution, and dichloromethane (Duksan Pure Chemicals) was used as solvent in the following ratio: 2 g CHX diacetate + 4 g ethyl cellulose + 200 mL dichloromethane

First, ethyl cellulose was completely dissolved in dichloromethane solvent using a stirrer for 24 h. Next, CHX diacetate was added to the solution.

**Preparation of elastomeric ligatures:**

Hypo-allergic latex-free orthodontic elastomeric ligatures (American Orthodontics) with an internal diameter of 0.045 inch and external diameter of 0.115 inch were rinsed in distilled water and dried at 60°C. The coating solution was sprayed on the ligatures five times. Each time, each side of the wire was sprayed twice. After the first spray, the ligature was dried for 5 min and then it was sprayed again. Next, elastomeric ligatures were dried at room temperature for 1 h, and three rings of each type of ligature (CHX-releasing and conventional ligatures) were cultured to ensure absence of S. mutans.

**Clinical procedure:**

In the first visit, baseline saliva samples were collected from both the test and control group patients. The patients were requested not to eat or drink for 2 h prior to sampling. The patients were requested to sit on a dental chair for 5 min, and 0.5 mL of unstimulated saliva was collected using a sterile syringe

and transferred into a microtube to determine the salivary count of S. mutans. Immediately after saliva collection, dental plaque sample was obtained from the second premolar site bilaterally using a sterile periodontal probe. The plaque sample was transferred into a microtube containing 1 mL saline. After sampling, the teeth underwent professional cleaning and the brackets were bonded. Roth brackets with a slot size of 0.022x0.028 inch were used in all patients. A minimum of 20 brackets were used for patients. The same type of composite and adhesive was used for bracket bonding in all patients. CHX-releasing ligatures were used for patients in the test group while conventional ligatures were used for control patients. The ligatures had similar color coding in both groups. Thus, the patients were blinded to the type of ligatures. The samples were placed next to dry ice and were sent to a microbiology lab in less than 2 h. Saliva samples were collected again at 7, 14, 21 and 28 days after placement of ligatures. On day 28, dental plaque samples were also collected in addition to saliva samples from the same site. Also, elastomeric ligatures were removed from the oral cavity using a sterile explorer and placed in 1 mL of saline and sent to the microbiology lab.

**Culture medium:**

To prepare the Mitis Salivarius Agar culture medium (Quelab, UK), 90 g of culture medium powder and 200 g of sucrose (Merck, Darmstadt, Germany) were dissolved in 1 L of distilled water and autoclave-sterilized at 110°C and 15 ibs/in$^2$ pressure for 15 min. After cooling to 50°C, 1 mL/L of 1% potassium tellurite (Merck, Darmstadt, Germany) and 0.2 µ/mL of bacitracin stock solution (previously sterilized by a syringe filter) were added to the culture medium. After mixing, 25 mL of culture medium was poured into sterile plates.

**Bacterial colony counting:**

Samples transferred to the microbiology lab were centrifuged and 10 serial dilutions were prepared using sterile serum. Of each dilution, 0.1 unit/mL was streak-cultured on Mitis Salivarius Agar culture medium. The culture plates were then incubated at 37°C for 48 h. To ensure that the counted colonies were S. mutans colonies, they were Gram-stained and catalase and Voges-Proskauer tests were also performed. Gram-staining revealed chains of Gram-positive cocci. The bacteria could not break down hydrogen peroxide and the catalase test result was negative. The Voges-Proskauer test result was positive and color change was observed. Thus, after the completion of incubation period and staining, the prominent, granulated, blue colonies of S. mutans were counted. In order to obtain the total number of bacteria present in the original sample, the number of counted colonies at each concentration was multiplied by the inverse of the dilution factor.

Number of colony forming units per milliliter (CFUs/mL) = number of colonies/(transferred volume x dilution factor) OR number of colonies x inverse of the dilution factor ($10^{-4}$) x inverse of the transferred volume (0.1 mL)

**Statistical analysis:**

Data were analyzed using SPSS version 25 (SPSS Inc., IL, USA). Normality of the data was evaluated using the Kolmogorov-Smirnov test. The Mann Whitney test was used to compare the salivary colony count between the two groups at different time points. The Friedman test was used to compare the colony count in the saliva samples separately in each group at 7, 14, 21 and 28 days. The Wilcoxon test was used to analyze the difference in salivary colony count at different time points. The Mann Whitney test was applied to compare the bacterial colony count in plaque samples between the two groups at different time points. The Wilcoxon test was applied to analyze the difference in bacterial count in plaque samples in each group at different time points. The bacterial colony count on elastomeric ligature samples was compared between the two groups at different time points using the Mann Whitney test.

**Results**

The Kolmogorov-Smirnov test revealed that data were not normally distributed in the test and control groups ($P<0.05$). Thus, non-parametric tests were applied for data analysis.

**Salivary count of S. mutans:**

Table 1 presents the salivary count of S. mutans at baseline and at 7, 14, 21 and 28 days after the intervention. The Mann-Whitney test revealed no significant difference in salivary count of S. mutans between the test and control groups at baseline (P=0.78). However, the salivary count of S. mutans started to increase in the control group and started to decrease in the test group during the course of study. The salivary count of S. mutans was significantly lower in the test group at 7, 14, 21 and 28 days (P<0.05).

**Table 1.** Salivary count of S. mutans at baseline and at 7, 14, 21 and 28 days after the intervention

| Time point | Control group | | Test group | | P value |
|---|---|---|---|---|---|
| | Mean | Std. deviation | Mean | Std. deviation | |
| Baseline | 1.08 | 0.49 | 1 | 0.55 | 0.78 |
| 7 days | 1.07 | 0.42 | 0.69 | 0.48 | 0.037 |
| 14 days | 1.14 | 0.47 | 0.39 | 0.32 | <0.001 |
| 21 days | 1.13 | 0.55 | 0.21 | 0.21 | <0.001 |
| 28 days | 1.23 | 0.61 | 0.13 | 0.12 | <0.001 |

*The mean and std. deviation values are based on $10^5$ CFUs.

Friedman test was then applied for within-group comparisons. It revealed no significant difference in salivary count of S. mutans in the control group during the one-month study period (P=0.061). However, this difference was significant in the test group (P<0.001). Wilcoxon test was then applied to analyze the difference in salivary count of S. mutans at different time points in the two groups. In the control group, the difference in this regard was not significant between different time points (P>0.05, Table 2). However, a significant reduction in S. mutans colony count was noted over time in the test group (P<0.05, Table 2).

**Table 2.** Results of Wilcoxon test for pairwise comparisons of salivary S. mutans colony count at different time points

| Time | Control group | Test group |
|---|---|---|
| Baseline-7 days | 0.865 | 0.001 |
| 7 days-14 days | 0.363 | 0.001 |
| 14 days-21 days | 0.691 | 0.001 |
| 21 days-28 days | 0.105 | 0.004 |
| Baseline-28 days | 0.088 | 0.001 |

**S. mutans count in dental plaque:**

Table 3 presents the S. mutans count in dental plaque samples collected at baseline and at 28 days. The Mann Whitney test was used to compare the plaque S. mutans count of the two groups, which revealed no significant difference between the test and control groups in this respect (P>0.05). Wilcoxon test revealed no significant increase in colony count in dental plaque at 28 days compared with baseline in the control group (P=0.910). In the test group, no significant reduction was noted in S. mutans colony count at 28 days compared with baseline (P=0.081).

**Table 3.** S. mutans colony count in dental plaque samples collected at baseline and at 28 days

| | Control group | | Test group | | P value |
|---|---|---|---|---|---|
| | Mean | Std. deviation | Mean | Std. deviation | |
| Baseline | 1.1 | 0.47 | 1 | 0.54 | 0.425 |
| 28 days | 1.11 | 0.51 | 0.97 | 0.54 | 0.400 |

*The mean and std. deviation values are based on $10^5$ CFUs.

**S. mutans count on elastomeric ligature samples:**

The mean count of S. mutans on elastomeric ligature samples was 0.51±0.48 ($x10^5$) CFUs in the test and 1.02±0.50 ($x10^5$) CFUs in the control group at 28 days. The Mann Whitney test revealed a significant difference in this respect between the two groups (P=0.003).

**Discussion**

This study aimed to assess the effect of CHX-releasing elastomeric ligatures on the number of S. mutans in the saliva and dental plaque and on elastomeric ligatures in orthodontic patients. The results showed that the salivary count of S. mutans was significantly lower in the test group at 7, 14, 21 and 28 days (P<0.05). The S. mutans count on the surface of CHX-releasing ligatures was significantly lower than that on the surface of conventional ligatures (P<0.05). However, no significant difference was noted in S. mutans count in plaque samples between the two groups (P>0.05). The salivary level of S. mutans significantly decreased during the course of study in the test group.

Masek et al. [29] showed that the salivary level of Lactobacillus and S. mutans significantly decreased 1 and 2 months after the application of CHX varnish three times a week. The salivary level of bacteria was significantly lower in the test group compared with the control group and after 2 months, the level of bacteria was lower than that at baseline. They suggested that CHX varnish can be applied every 3 months to exert maximum antibacterial effects. Although their methodology was different from ours and they used CHX varnish, their results were in agreement with our findings since we also noticed a significant reduction in S. mutans count of the saliva in the test group over time. Twetman et al. [30] found that application of CHX varnish significantly decreased the level of S. mutans at 1 week and 1 month, but the difference was no longer significant after 3 and 6 months. A systematic review by Tange et al. [31] evaluated the antibacterial effects of CHX varnish on fixed orthodontic patients and concluded that CHX varnish was effective on S. mutans only after 3 to 4 weeks. Although their results were in line with our findings, monthly application of CHX varnish is not cost-effective during the 2-year treatment period and is also time consuming. Khan and Antony [32] reported that CHX varnish effectively decreased plaque accumulation and improved gingival health within 3 months while plaque and gingival index increased in the control group and then decreased at 6 months. Despite this reduction, at 6 months, the plaque index in the control group was higher than the baseline value. Similarly, Alexander [33] reported an increase in plaque index during the course of orthodontic treatment. We did not assess the plaque index in this study; however, the count of S. mutans in dental plaque increased in the control group, although insignificantly.

Liptak et al. [34] assessed the effect of monthly application of CHX gel on S. mutans and Lactobacillus counts in the saliva and plaque during a 6-month period. They reported that the reduction in S. mutans count of dental plaque was significant after 2 months.

In the present study, plaque samples were collected after 28 days. However, CHX released from the ligatures might have required a longer period of time to exert its effect on bacterial plaque. Thus, studies with longer follow-ups are required to better elucidate this topic. On the other hand, the biofilm structure of plaque may inhibit the penetration and infiltration of antibacterial agents. The superficial bacteria proliferate slowly and show a new phenotype, which is responsible for decreased sensitivity and increased resistance to plaque-inhibiting agents.

The efficacy of fluoride-releasing elastomeric ligatures has been extensively studied in orthodontic patients. Miura et al. [13] reported that use of elastomeric ligatures releasing fluoride in orthodontic patients did not cause a significant change in the count of S. mutans in the saliva or dental plaque. Benson et al. [35] showed that fluoride-releasing elastomers were not clinically effective for reduction of S. mutans count. Similar results were reported by Doherty et al [36].

Addition of fluoride or CHX to elastomeric ligatures seems logical because by doing so, antibacterial agent is easily delivered to the desired site. Also, elastics are constantly replaced and provide a fresh source of antibacterial agent. However, addition of fluoride did not show promising results [35,36]. But, our study demonstrated that addition of CHX to elastomeric ligatures caused a significant reduction in salivary count of S. mutans. Jeon et al. [23,28] reported that coating of elastics did not affect their physical properties.

Addition of fluoride or CHX to orthodontic bonding agents is another suggested strategy to benefit from their cariostatic and antimicrobial property in orthodontic patients. However, it does not seem ideal since the fluoride release potential would not last long [37]. Some fluoride-containing cements such as glass ionomers have the recharge potential and can not only release, but also uptake fluoride [38]. However, it is not known whether the level of fluoride released by these cements is adequately high during the entire course of orthodontic treatment to prevent WSLs. In general, the results of adding fluoride to composite resins have not been promising [16]. Despite the cariostatic effects of glass ionomers, they have significantly lower strength than composite resins and have a high rate of debonding [39]. Chung et al. [40] showed that a primer containing CHX had strong antimicrobial activity and did not significantly affect the bond strength. However, CHX-containing adhesives are only applied for bracket bonding, and their antibacterial agent reservoir would not last long; whereas, CHX-releasing elastomeric ligatures are replaced on a monthly basis and provide a fresh source of CHX. In this study, we asked patients not to use fluoride- or CHX-containing gels or mouthwashes during the study period to prevent their confounding effects on the results. None of our patients showed any side effect related to the use of CHX, which is probably due to the use of relatively low concentration of CHX in the coating. However, our study was conducted during one-month period and studies with longer follow-ups are required to cast a final judgment in this respect. Future studies are required to assess the antibacterial efficacy of elastomeric ligatures with CHX added to their polymeric structure. By doing so, we may be able to ensure the sustained release of CHX over a longer period of time.

## Conclusion

CHX-releasing elastomeric ligatures can significantly decrease the salivary count of S. mutans in orthodontic patients.

## Reference

1. Ren Y, Jongsma MA, Mei L, van der Mei HC, Busscher HJ. Orthodontic treatment with fixed appliances and biofilm formation—a potential public health threat?. Clinical oral investigations. 2014 Sep 1;18(7):1711-8.

2. Sukontapatipark W, El-Agroudi MA, Selliseth NJ, Thunold K, Selvig KA. Bacterial colonization associated with fixed orthodontic appliances. A scanning electron microscopy study. The European Journal of Orthodontics. 2001 Oct 1;23(5):475-84.

3. Mitchell L. Decalcification during orthodontic treatment with fixed appliances—an overview. British Journal of Orthodontics. 1992 Aug 1;19(3):199-205.

4.Graber LW, Vanarsdall RL, Vig KW, Huang GJ. Orthodontics-E-Book: current principles and techniques. Elsevier Health Sciences; 2016 Jul 15.

5. Al Maaitah EF, Adeyemi AA, Higham SM, Pender N, Harrison JE. Factors affecting demineralization during orthodontic treatment: a post-hoc analysis of RCT recruits. American Journal of Orthodontics and Dentofacial Orthopedics. 2011 Feb 1;139(2):181-91.

6.Jing D, Hao J, Shen Y, Tang G, Lei L, Zhao Z. Effect of fixed orthodontic treatment on oral microbiota and salivary proteins. Experimental and therapeutic medicine. 2019 May 1;17(5):4237-43.

7. Chang HS, Walsh LJ, Freer TJ. The effect of orthodontic treatment on salivary flow, pH, buffer capacity, and levels of mutans streptococci and lacto bacilli. Australian orthodontic journal. 1999 Apr;15(4):229.

8.Klein MI, Hwang G, Santos PH, Campanella OH, Koo H. Streptococcus mutans-derived extracellular matrix in cariogenic oral biofilms. Frontiers in cellular and infection microbiology. 2015 Feb 13;5:10.

9.Øgaard B, Rølla G, Arends J. Orthodontic appliances and enamel demineralization: Part 1. Lesion development. American Journal of Orthodontics and Dentofacial Orthopedics. 1988 Jul 1;94(1):68-73.

10.Acharya S, Goyal A, Utreja AK, Mohanty U. Effect of three different motivational techniques on oral hygiene and gingival health of patients undergoing multibracketed orthodontics. The Angle Orthodontist. 2011 May 25;81(5):884-8.

11.Lalic M, Aleksic E, Gajic M, Milic J, Malesevic D. Does oral health counseling effectively improve oral hygiene of orthodontic patients?. European journal of paediatric dentistry: official journal of European Academy of Paediatric Dentistry. 2012 Sep;13(3):181-6.

12.Hadler-Olsen S, Sandvik K, El-Agroudi MA, Øgaard B. The incidence of caries and white spot lesions in orthodontically treated adolescents with a comprehensive caries prophylactic regimen—a prospective study. The European Journal of Orthodontics. 2011 Jul 12;34(5):633-9.

13.Miura KK, Ito IY, Enoki C, Elias AM, Matsumoto MA. Anticariogenic effect of fluoride-releasing elastomers in orthodontic patients. Brazilian oral research. 2007 Sep;21(3):228-33.

14.Liang K, Weir MD, Reynolds MA, Zhou X, Li J, Xu HH. Poly (amido amine) and nano-calcium phosphate bonding agent to remineralize tooth dentin in cyclic artificial saliva/lactic acid. Materials Science and Engineering: C. 2017 Mar 1;72:7-17.

15.Pereira-Cenci T, Cenci MS, Fedorowicz Z, Azevedo M. Antibacterial agents in composite restorations for the prevention of dental caries. Cochrane Database of Systematic Reviews. 2013(12).

16.Turner PJ. The clinical evaluation of a fluoride-containing orthodontic bonding material. British journal of orthodontics. 1993 Nov;20(4):307-13.

17.Trimpeneers LM, Dermaut LR. A clinical evaluation of the effectiveness of a fluoride-releasing visible light-activated bonding system to reduce demineralization around orthodontic brackets. American journal of orthodontics and dentofacial orthopedics. 1996 Aug 1;110(2):218-22.

18.Banks PA, Burn A, O'Brien K. A clinical evaluation of the effectiveness of including fluoride into an orthodontic bonding adhesive. European journal of orthodontics. 1997 Aug 1;19(4):391-5.

19.Borzabadi-Farahani A, Borzabadi-Farahani A, Eslamipour F. Orthodontic treatment needs in an urban Iranian population, an epidemiological study of 11-14 year old children. European Journal of Paediatric Dentistry. 2009 Jun 1;10(2):69.

20.Prasanth M. Antimicrobial efficacy of different toothpastes and mouthrinses: an in vitro study. Dental research journal. 2011;8(2):85.

21.Hugo WB, Longworth AR. The effect of chlorhexidine on the electrophoretic mobility, cytoplasmic constituents, dehydrogenase activity and cell walls of Escherichia coli and Staphylococcus aureus. Journal of Pharmacy and Pharmacology. 1966 Sep;18(9):569-78.

22.Van Strijp AJ, Gerardu VA, Buijs MJ, Van Loveren C, Ten Cate JM. Chlorhexidine Efficacy in Preventing Lesion Formation in Enamel and Dentine. Caries research. 2008;42(6):460-5.

23.Jeon HS, Choi CH, Kang SM, Kwon HK, Kim BI. Chlorhexidine-releasing orthodontic elastomerics. Dental materials journal. 2015 May 28:2014-16.

24.Maruo IT, Rosa EA, Maruo H, Tanaka O, Guariza Filho O, Ignácio SA, Camargo ES. Effect of chlorhexidine mouth rinse on Streptococci counts of tooth-tissue-borne palatal expander biofilm. Orthodontics & craniofacial research. 2008 Aug;11(3):136-42.

25.Verraedt E, Pendela M, Adams E, Hoogmartens J, Martens JA. Controlled release of chlorhexidine from amorphous microporous silica. Journal of Controlled Release. 2010 Feb 25;142(1):47-52.

26.Verraedt E, Braem A, Chaudhari A, Thevissen K, Adams E, Van Mellaert L, Cammue BP, Duyck J, Anné J, Vleugels J, Martens JA. Controlled release of chlorhexidine antiseptic from microporous amorphous silica applied in open porosity of an implant surface. International journal of pharmaceutics. 2011 Oct 31;419(1-2):28-32.

27.Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM. Polymeric systems for controlled drug release. Chemical reviews. 1999 Nov 10;99(11):3181-98.

28.Jeon HS, Jung EH, Kang SM, Lee ES, Lee JW, KIM BI. Improving the efficacy of chlorhexidine-releasing elastomerics using a layer-by-layer coating technique. Dental materials journal. 2017 Jul 25;36(4):476-81.

29.Mašek I, Matošević D, Jurić H, Meštrović S. Antimicrobial effects of chlorhexidine in orthodontic patients. Acta stomatologica Croatica. 2008 Mar 15;42(1):41-8.

30.Twetman S, Hallgren A, Petersson LG. Effect of an antibacterial varnish on mutans streptococci in plaque from enamel adjacent to orthodontic appliances. Caries research. 1995;29(3):188-91.

31.Tang X, Sensat ML, Stoltenberg JL. The antimicrobial effect of chlorhexidine varnish on mutans streptococci in patients with fixed orthodontic appliances: a systematic review of clinical efficacy. International journal of dental hygiene. 2016 Feb;14(1):53-61.

32.Khan R, Antony V. A Randomized Clinical Trial Evaluating the Efficacy of Chlorhexidine Varnish on Streptococcus mutans in Plaque and the Gingival Status in Patients Undergoing Orthodontic Treatment. The Orthodontic Cyber Journal. 2010:2-14.

33.Alexander SA. Effects of orthodontic attachments on the gingival health of permanent second molars. American Journal of Orthodontics and Dentofacial Orthopedics. 1991 Oct 1;100(4):337-40.

34.Lipták L, Szabó K, Nagy G, Márton S, Madléna M. Microbiological changes and caries-preventive effect of an innovative varnish containing chlorhexidine in orthodontic patients. Caries research. 2018;52(4):272-8.

35.Benson PE, Douglas CI, Martin MV. Fluoridated elastomers: effect on the microbiology of plaque. American journal of orthodontics and dentofacial orthopedics. 2004 Sep 1;126(3):325-30.

36.Doherty UB, Benson PE, Higham SM. Fluoride-releasing elastomeric ligatures assessed with the in situ caries model. The European Journal of Orthodontics. 2002 Aug 1;24(4):371-8.

37.Geiger AM, Gorelick L, Gwinnett AJ, Benson BJ. Reducing white spot lesions in orthodontic populations with fluoride rinsing. American Journal of Orthodontics and Dentofacial Orthopedics. 1992 May 1;101(5):403-7.

38.Marcusson A, Norevall LL, Persson M. White spot reduction when using glass ionomer cement for bonding in orthodontics: a longitudinal and comparative study. European Journal of Orthodontics. 1997 Jun 1;19(3):233-42.

39.Norevall LI, Marcusson A, Persson M. A clinical evaluation of a glass ionomer cement as an orthodontic bonding adhesive compared with an acrylic resin. European Journal of Orthodontics. 1996 Aug 1;18(4):373-84.

40.Chung SH, Cho S, Kim K, Lim BS, Ahn SJ. Antimicrobial and physical characteristics of orthodontic primers containing antimicrobial agents. The Angle Orthodontist. 2016 Sep 6;87(2):307-12.