

## Original Article

# Evaluation of the effects of fluoride mouth rinse and varnish on the early biofilm formation of *Streptococcus mutans* in two types of orthodontic adhesive resins: An *in vitro* study

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## ABSTRACT

**Background:** The aim of the present study is to compare the antibacterial effect of fluoride mouth rinse and fluoride varnish on the primary biofilm formation of *Streptococcus mutans* (*S. mutans*) in two types of orthodontic adhesives.

**Materials and Methods:** This is an *in vitro* study in which forty composite discs of Transbond XT and Lightbond were divided randomly into 4 groups: Group 1: Control group (not treated with fluoride), Group 2: Rinsed by 0.2% fluoride mouth rinse, Group 3: Rinsed by 0.05% fluoride mouth rinse, and Group 4: Treated by varnish fluoride. Then each group was placed in *S. mutans* suspension. Bacterial suspension from each treatment was subcultured onto the surface of Mueller–Hinton agar plates, and bacterial growth was assessed. The results were analyzed by analysis of variance test and Scheffé test was run to compute the binary groups ( $P < 0.05$ ).

**Results:** There was a statistically significant reduction in the viability of *S. mutans* in treated groups by fluoride but no significant difference between two types of composites.

**Conclusion:** The results of this study demonstrated that *S. mutans* colonies were sensitive to fluoride and their most effective form was varnish. There was no significant difference in early biofilm formation of *S. mutans* in two types of orthodontic adhesive resins Transbond XT and Lightbond.

**Key Words:** Composite resins, fluoride, *Streptococcus mutans*

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## INTRODUCTION

Adequate hygiene is difficult for orthodontic patients due to orthodontic appliances. For this reason, these appliances have the ability to change the microbial environment of the mouth. Following these changes, the risk of dental caries also increases.<sup>[1]</sup>

Dental caries is a process that is caused by the acids produced by oral bacteria.<sup>[2,3]</sup>

These bacteria, the most common of which are *Streptococcus mutans* (*S. mutans*), produce acid with the carbohydrate metabolites in the mouth, followed by oral contractions. In this situation, the demineralization and remineralization balances are fluttering, and more demineralization takes place, which eventually leads to White Spot Lesions (WSL).<sup>[4-6]</sup>

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Gorelick *et al.* stated that 49.6% of patients undergoing orthodontic treatment had at least one WSL at the end of the treatment.<sup>[7]</sup>

In another study, they investigated the effect of fluoride on the formation of biofilm *S. mutans* and found that fluoride resulted in 90% decrease in the formation of biofilm *S. mutans*. The experiment showed that fluoride, without affecting the life of the *S. mutans*, reduced the formation of its biofilm.<sup>[8]</sup>

At low concentration of fluoride found in toothpaste, acceptable results were obtained.<sup>[9,10]</sup>

Varnish fluoride, in addition to the inhibitory effect on the adhesion of *S. mutan*, causes biofilm acidogenicity to change, these results could be due to the release of fluoride ions.<sup>[11]</sup>

Fluoride mouth rinse leads to demineralization-reminerization balance toward remineralization and has an inhibitory effect on the tooth decay.<sup>[12]</sup>

Sonesson *et al.* (2020) showed fluoride varnish formula containing 1.5% ammonium fluoride in preventing WSLs in adolescents undergoing multi-bracket orthodontic treatment.<sup>[13]</sup>

Normally, in patients with a high risk of decay, it is advisable to use a fluoride mouth rinse together with a toothpaste because it is easy to use and maintains a high concentration of fluoride in the mouth.<sup>[14]</sup>

O'Reilly and Featherstone showed that the use of oral mucosa with 0.05% fluoride mouth rinse in addition to fluoride toothpaste prevents demineralization.<sup>[15]</sup>

Enerbäck *et al.* showed that the everyday use of high-fluoride toothpaste (5000 ppm F) or mouth rinse (0.2% NaF) in combination with 1450 ppm F toothpaste is recommended to reduce caries risk during orthodontic treatment, as compared to using only ordinary toothpaste.<sup>[16]</sup>

Since orthodontic patients are at high risk for decay, in addition to fluoride toothpaste, fluoride mouth rinse should be also be used. According to studies, the use of fluoride bonding agents has shown a significant reduction in the amount of tooth decalcification.<sup>[17,18]</sup>

Since the common adhesive resins used in orthodontics are fluoride-free, the present study is designed to investigate the antibacterial effect of fluoride varnish and fluoride mouth rinse on the early biofilm formation of *S. mutans*. No study has been done so far, neither to compare two concentration

of fluoride mouth rinse nor biofilm formation of *S. mutans* in two types of orthodontic adhesives (Transbond XT (3M Unitek, Monrovia, Calif) and Lightbond (Reliance Orthodontic Products, Itasca, Ill, USA).

The aim of the present study is to compare the antibacterial effect of fluoride mouth rinse and fluoride varnish on the primary biofilm formation of *S. mutans* in two types of orthodontic adhesives.

## MATERIALS AND METHODS

This is an *in vitro* study in which forty composite discs of Transbond XT (3M Unitek, Monrovia, Calif) and Lightbond (Reliance Orthodontic Products, Itasca, Ill, USA) with the dimensions of 6 mm in diameter and 1 mm in depth were prepared. For the preparation of the samples, the PAPCO sheets were punched at the above size and put them on glass slab, then adhesives were inserted into the hole and another glass slab put on them and cured. These composite discs were cured for 20 s (according to the manufacturer's recommendations). The samples were divided randomly into 4 groups of 10 composites; each group including equal number of both types of composites. The final groups were as below:

- Group 1: Control group (not treated with fluoride)
- Group 2: To assess the effect of 0.2% fluoride mouth rinse (Fluoride–Behsa mouth rinse, Iran) on the primary biofilm formation of *S. mutans*
- Group 3: To assess the effect of 0.05% fluoride mouth rinse (Fluoride–Behsa mouth rinse, Iran) on the primary biofilm formation of *S. mutans*
- Group 4: To assess the effect of fluoride varnish (Fluor protector Ivoclar Vivadent) on the primary biofilm formation of *S. mutans*.

Composite bonding materials were selected according to the frequency of use in orthodontic procedures.

All discs were sterilized in autoclave at 121°C and 15 pounds pressure for 15 min.

### Assessment of primary biofilm formation

For preparation of fresh bacterial culture, standard *S. mutans* strain (PTCC 1683) was inoculated in a culture environment enriched with 5% Brain Heart Infusion Agar (BHI) and incubated for 24 h at 37°C. Then, some fresh colonies were transferred to 5 cc sterile physiologic serum, and a suspension equivalent to the half Mcfarland opacity was obtained. To ensure the turbidity of  $1/5 \times 10^8$ , the bacterium was used in

a milliliter of a spectrophotometer. To examine the bacterial adhesion to the discs, sterile BHI culture media and 24-well microplates were used. So, each well contained one disc sample, 1.5 cc BHI Broth and 0.1cc *S. mutans* suspension. The plates were maintained at 37°C in CO<sub>2</sub> incubator for 6 h. Then, the samples were removed from the microplates and washed twice with sterile phosphate-buffered saline (pH = 7.2) to remove the loosely bound materials. Next, they were inserted in a tube with 3cc sterile physiologic serum and then placed in an ultrasonic bath at 25 Hz for 7–10 min to disperse the biofilms. An aliquot (0.1 ml) of the dispersed solution was serially diluted and plated onto BHI agar plates. Then, the number of colony-forming units per milliliter (CFU/ml) was determined.

It should be mentioned that in Group 2, before inserting the discs in microplates, they were immersed in 5 cc of 0.2% fluoride mouth rinse for 1 min, after which the biofilm formation was evaluated and compared with control group.

In Group 3, before inserting the discs in microplates, they were immersed in 5 cc of 0.05% fluoride mouth rinse for 1 min, following which the biofilm formation was evaluated and compared with control group.

In Group 4, before inserting the discs in microplates, one thin layer of varnish was applied to the discs and dried at 59°C for 24 h.<sup>[11]</sup> Then, the biofilm formation was evaluated and compared with control group.

### Statistical analysis

Data were analyzed by SPSS-17 software (653e19d84509a40a2dfe California USA). Tables and indices were prepared, and two-way analysis of variance test was used for analyses. Scheffé test was run to compute the binary groups.

## RESULTS

In Transbond XT (3M Unitek, Monrovia, Calif) groups, which were treated with fluoride mouth rinse 0.2% and fluoride varnish, the reduced viability of *S. mutans* was statistically significant ( $P < 0/05$ ). There was no significant difference between control (Group 1) and fluoride mouth rinse 0.05% (Group 3). The highest amount of *S. mutans* colonies in the treated groups was observed in fluoride mouth rinse 0.05% (Group 3) and the lowest amount was found in fluoride varnish (Group 4) [Table 1].

In Lightbond (Reliance Orthodontic Products, Itasca, Ill, USA) groups, which were treated with fluoride

mouth rinse 0.2% and 0.05% and fluoride varnish, the reduction of *S. mutant* colonies was statistically significant ( $P < 0/05$ ). The highest amount of *S. mutans* colonies in the treated groups occurred in fluoride mouth rinse 0.05% (Group 3) and the lowest amount occurred in fluoride varnish (Group 4) [Table 2].

In two-by-two comparison of fluoride-treated groups in terms of the number of *S. mutans* colonies, in both groups of studied resin, results showed the reduction of *S. mutant* colonies in Transbond XT (3M Unitek, Monrovia, Calif, USA) groups treated fluoride mouth rinse 0.2% was significantly higher than those of the groups treated with fluoride mouth rinse 0.05%. Fluoride varnish groups showed that, in comparison to both concentrations of mouth rinse, there was a significant reduction in the number of mutant colonies [Table 3].

The reduction of *S. mutant* colonies in Lightbond (Reliance Orthodontic Products, Itasca, Ill, USA) groups, which were treated with fluoride mouth rinse 0.2%, was significantly higher than those of the groups treated with fluoride mouth rinse 0.05%. Fluoride varnish groups showed that, in comparison to both concentrations of mouth rinse, there was a significant reduction in the number of mutant colonies [Table 3].

The results showed no significant difference in early biofilm formation of *S. mutans* in the two

**Table 1: Means±standard deviation and P value of the number of log<sub>10</sub> of *Streptococcus mutant* colony obtained for different groups of transbond XT composite bonding**

Groups	Mean±SD	P
Control	3±0.00	-
Fluoride mouth rinse 0.05%	2.93±2.31	0.143
Fluoride mouth rinse 0.2%	2.71±2.22	0.000*
Fluoride varnish	0.95±1.20	0.000*

\*Significant difference between study groups and control ( $P < 0.05$ ). SD: Standard deviation

**Table 2: Means±standard deviation and P value of the number of log<sub>10</sub> of *Streptococcus mutant* colony obtained for different groups of reliance composite bonding**

Groups	Mean±SD	P
Control	3±0.00	-
Fluoride mouth rinse 0.05%	2.84±2.44	0.023*
Fluoride mouth rinse 0.2%	2.58±2.20	0.000*
Fluoride varnish	0±0.50	0.000*

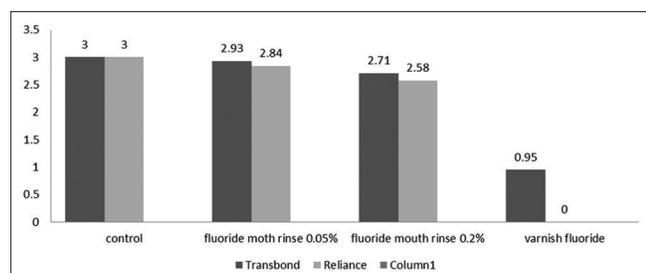
\*Significant difference between study groups and control ( $P < 0.05$ ). SD: Standard deviation

types of orthodontic adhesive resins (Transbond XT [3M Unitek, Monrovia, Calif, USA] and Lightbond [Reliance Orthodontic Products, Itasca, Ill, USA]) ( $P = 0.269$ ).

Figure 1 shows the effect of fluoride on CFU of *S. mutans* in two types of adhesive resins. As indicated, the most effective form of fluoride used on early biofilm formation of *S. mutans* in both adhesive resins is fluoride varnish (Group 4) and the least effective is fluoride mouth rinse 0.05% (Group 3). In general, the highest colony count of *S. mutans* was found in Lightbond (Reliance Orthodontic Products, Itasca, Ill, USA) groups, which were treated with fluoride varnish, and the lowest count was observed in Transbond XT (3M Unitek, Monrovia, Calif, USA) groups, which were treated with fluoride mouth rinse 0.05%.

## DISCUSSION

Dental caries is one of the most common infectious diseases caused by biofilm accumulation of bacteria.<sup>[19,20]</sup>



**Figure 1:** The effect of fluoride on CFU of *S. mutans* in two types of adhesive resins

**Table 3: Two-by-two comparison of study groups**

Groups	P
Transbond	
Fluoride mouth rinse 0.05%	0.003*
Fluoride mouth rinse 0.2%	
Fluoride mouth rinse 0.05%	0.000*
Fluoride varnish	
Fluoride mouth rinse 0.2%	0.000*
Fluoride varnish	
Reliance	
Fluoride mouth rinse 0.05%	0.015*
Fluoride mouth rinse 0.2%	
Fluoride mouth rinse 0.05%	0.000*
Fluoride varnish	
Fluoride mouth rinse 0.2%	0.000*
Fluoride varnish	

\*Significant difference between the two groups compared ( $P < 0.05$ )

Following the bacterial adhesion to the teeth surface, a biofilm can be created which can cause tooth decay. Therefore, by controlling and reducing the biofilm accumulation, we can prevent caries. By using fluoride, biofilm can be prevented on the surface of the teeth. Hence, this fluoride can be used in caries prevention approaches.<sup>[21]</sup>

The use of various forms of topical fluoride (gel, mouth rinse, varnish) is helpful in decontamination in patients undergoing an orthodontic treatment that is likely to develop WSL.<sup>[22,23]</sup>

Since the common composites used in orthodontic treatment are light cure and the common light cure composites are Transbond XT and Lightbond, we decided to select the composite discs of these two types and since orthodontic patients are at high risk of caries and need to use fluoride during treatment, we examined the effect of antibacterial fluoride on the early formation of the biofilm of *S. mutans* in these two composites and to find the most effective form of fluoride in clinical setting, by placing the composites in variety of fluoride forms. Because braces were bonded to enamel using composite, we choose two types of common composite to prepare the discs to simulate the biofilm formation condition and evaluate the formation of *S. mutans* around composite discs. As expressed in introduction, the aim of this study was to compare the antibacterial effect of fluoride mouth rinse and fluoride varnish on the primary biofilm formation of *S. mutans* in two types of orthodontic adhesives. Therefore, composite discs were fabricated to evaluate their suitability for antibacterial effect. Fluoride varnishes are clinically effective in the prevention of dental caries.<sup>[24,25]</sup> Due to their effectiveness and safety, their use has increased among the dental community.<sup>[26]</sup>

Chau *et al.* concluded that fluoride varnish application can affect cariogenic biofilm formation.<sup>[11]</sup>

Songsiripradubboon *et al.* stated that the rinsing frequency of NaF mouth rinse, when used with fluoride toothpaste, also affects the remineralization.<sup>[27]</sup> The findings of the present study showed that mean *S. mutans* bacterial colony count in all fluoride-treated groups was significantly reduced except in Transbond XT (3M Unitek, Monrovia, Calif, USA) groups, which were treated with fluoride mouth rinse 0.05%. In the present study, maybe because of low fluoride concentration and that the number of unreacted methacrylate groups after curing, in the Transbond

XT (3M Unitek, Monrovia, Calif, USA) is low.<sup>[28]</sup> The fluoride varnish had the greatest effect in reducing the early biofilm formation of mutans. Then, fluoride mouth rinse 0.2% and fluoride mouth rinse 0.05% had the lowest reduction (in both orthodontic adhesive resin).

Jane and Koch *et al.* compared fluoride varnish with fluoride mouth rinse 0.2% and suggested that fluoride varnish was more effective in preventing decay, which confirmed the results of this study.<sup>[29,30]</sup>

Further, Marinho *et al.* compared the effectiveness of two types of topical fluorides in preventing dental caries and reported no significant difference between fluoride varnish and mouth rinse 0.2%, which is contrary to the results of this study.<sup>[9]</sup>

The reason for the higher effect of fluoride varnish may be because salivary fluoride concentration is more durable and lasts longer after using varnish.<sup>[31]</sup>

In the present study, there was no significant difference in early biofilm formation of *S. mutans* between the two types of orthodontic adhesive resins Transbond XT (3M Unitek, Monrovia, Calif, USA) and Lightbond (Reliance Orthodontic Products, Itasca, Ill, USA). Some studies have confirmed our results.<sup>[32,33]</sup>

## CONCLUSION

The results of this study demonstrated that *S. mutans* colonies were sensitive to fluoride and their most effective form was varnish. There was no significant difference in early biofilm formation of *S. mutans* in two types of orthodontic adhesive resins Transbond XT (3M Unitek, Monrovia, Calif, USA) and Lightbond (Reliance Orthodontic Products, Itasca, Ill, USA).

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### Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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