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Quantitative Determination of Adhesion Patterns of Cariogenic Streptococci to Various Orthodontic Adhesives

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ABSTRACT

Objective: To investigate the adhesion of various cariogenic streptococci to orthodontic adhesives.

Materials and Methods: Five light-cure orthodontic adhesives (one fluoride-releasing composite, three non–fluoride-releasing composites, and one resin-modified glass ionomer cement) were used. The adhesive type, bacterial strain, incubation time, and saliva coating were studied. Thirty specimens of each adhesive were incubated with unstimulated whole saliva or phosphate-buffered saline for 2 hours. Binding assays were then performed by incubating tritium-labeled streptococci with the adhesives for 3 or 6 hours.

Results: The results showed a characteristic adhesion pattern according to the type of bacterial strains used. *Streptococcus mutans* LM7 showed the highest amount of adhesion, whereas *S sobrinus* B13 showed the lowest amount of adhesion. The cariogenic streptococci adhered to the glass ionomer significantly more than to the composites, whereas there was no significant difference in the adhesion amount among the four composites. The extended incubation time significantly increased bacterial adhesion. However, saliva coating did not significantly alter adhesion patterns of cariogenic streptococci.

Conclusions: This study suggests that cariogenic streptococci can adhere diversely according to adhesive type and that the adhesion of the cariogenic streptococci is not influenced by its fluoride-releasing properties.

KEY WORDS: Adhesion, Cariogenic streptococci, Orthodontic adhesive.

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INTRODUCTION Return to TOC

Enamel demineralization is a commonly recognized complication of orthodontic treatment with a fixed orthodontic appliance. The enamel demineralization is caused by organic acids produced mainly by mutans streptococci (MS), which have been shown to be the prime causative organisms of dental caries.[1] The placement of the fixed orthodontic appliance leads to an increase in the volume and number of MS within dental plaque.[2,3] Elevated levels of MS fall back to normal after removal of the appliance.[3] Among MS, *Streptococcus mutans* and *S sobrinus* are closely associated with enamel demineralization, and an increase in their number after the placement of orthodontic appliances has been reported.[4]

Clinical observation has indicated that the most common sites for demineralization are at the junction between the bonding adhesive and the enamel.[5,6] In particular, the orthodontic adhesives remaining on the enamel surface around the bracket are known to be risk factors for predisposition to enamel demineralization because the rough adhesive surface can provide a site for the rapid attachment and growth of oral microorganisms.[5,7] The adhesion of bacteria to surfaces forms an important initial stage in dental plaque formation and enamel demineralization.

Many orthodontic adhesives are commercially available. Composite and glass ionomer are the two main classes of orthodontic bonding adhesives. Although their physical properties, surface characteristics, and fluoride-releasing capacities have been extensively studied, their biologic properties associated with adhesion of cariogenic streptococci have not been well investigated. Differences in bacterial adhesion to the different orthodontic adhesives may be expected because of their different characteristics and the release of incorporated fluoride. In particular, glass ionomers have demonstrated an inhibitory effect on growth or adhesion of oral bacteria because of their fluoride-releasing properties.[8,9] However, the effect of glass ionomer adhesive on the adhesion of cariogenic bacteria has not been directly compared with that of composite adhesives. The purpose of this study was to observe the amount of cariogenic streptococci adhesion to various orthodontic adhesives and to compare the effect of fluoride release on the adhesion amount regarding the type of bacteria, incubation time, and saliva coating.

MATERIALS AND METHODS Return to TOC

Preparation of Bonding Adhesives

Five light-cure orthodontic bonding adhesives were selected, consisting of three non–fluoride-releasing composites, one fluoride-releasing composite, and one resin-modified glass ionomer cement (RMGI) (Table 1). Specimens were prepared with Teflon templates with 3.0-mm wide and 2.0-mm deep holes. Template plates were positioned on top of glass slides. Each bonding material was placed into the holes until the materials became flush with the top of the templates. A second slide was placed on top, pushed down to ensure flat dorsal surfaces, and then gently removed. All materials were handled according to the manufacturers' instructions and were light cured for 40 seconds (20 seconds from the top and 20 seconds from the bottom).

Saliva Collection

Saliva was collected from a 33-year-old man of good oral health who had refrained from eating, drinking, and brushing for at least 2 hours before saliva collection. This volunteer had no acute dental caries and periodontal lesions. Saliva collection was performed from 7:00 AM to 9:00 AM to minimize the effects of diurnal variability in salivary composition. Unstimulated whole saliva (UWS) was collected in a chilled sterile tube by a spitting method. The saliva sample was centrifuged at 3500 × *g* for 5 minutes to remove any cellular debris. The resulting supernatants were used immediately for the pellicle formation and bacterial adhesion assays.

Radioactive Labeling and Preparation of Cariogenic Streptococci

S mutans strains LM7 and OMZ65 and *S sobrinus* strains 6715 and B13 were used. The bacteria were stored at −70°C in Trypticase (GIBCO, Grand Island, NY) with 3% yeast extract (TYE) broth containing 40% glycerol. Radiolabeling was performed by incubating a loop of bacteria in 10 mL of TYE broth containing 50 µCi [^{3}H] thymidine ([*methyl*-^{3}H] thymidine, Amersham Pharmacia Biotech, Piscataway, NJ) for 16 hours anaerobically at 37°C. The tritium-labeled bacteria were harvested by centrifugation at 3500 × *g* for 5 minutes and washed in Hank's Balanced Salt Solution (GIBCO) supplemented with 4.0 mmol $NaHCO_3$, 1.3 mmol $CaCl_2$, 0.8 mmol $MgCl_2$, and 0.5% bovine serum albumin (HBSS-BSA, pH 7.2). After being washed twice, pellets were resuspended in HBSS-BSA and adjusted to a final concentration of 5×10^8 cells per milliliter at A_{660} with a Petroff-Hauser cell counter (Hauser Scientific Partnership, Horsham, Pa).

Adhesion of Streptococci to Orthodontic Bonding Adhesives

Thirty specimens of each adhesive were incubated in 2.0 mL of UWS with agitation for 2 hours at room temperature. For negative control tests, the same procedure was performed with sterile phosphate-buffered saline (PBS, pH 7.2) instead of UWS. After being washed three times in PBS, the specimens were incubated in 2.0 mL of HBSS-BSA containing 1×10^9 tritium-labeled bacteria with agitation for either 3 or 6 hours at 37°C. The specimens were then washed three times with HBSS-BSA and transferred to scintillation vials. The radiolabeled bacteria were dislodged from the specimens by incubation with 300 µL of 8 M urea, 1.0 M NaCl, and 1% sodium dodecyl sulfate with agitation for 1 hour at 37°C.[10] Then, 3.5 mL of scintillation cocktail was added and the number of adherent cells was determined with a Beckman LS-5000TA liquid scintillation counter (Beckman Instruments, Fullerton, Calif). The radioactive counts were divided by the total counts per minute of the bacterial suspension solution, and the amount of the cariogenic streptococci adhesion is defined as the percentage adhesion. All test samples were counted in triplicate in each experiment, and each experiment was repeated six times.

A four-way factorial analysis of variance (ANOVA) was used to analyze the adhesion amount and interaction effects of the cariogenic streptococci with respect to the strains, adhesive type, incubation time, and saliva coating. Multiple comparisons were done by the Bonferroni *t*-tests at a significance level of $\alpha = 0.05$.

RESULTS Return to TOC

Tables 2 and 3 present the amount of cariogenic streptococci adhesion with respect to bacterial strain, adhesive type, incubation time, and saliva coating. The results of four-way ANOVA indicate that bacterial strain, adhesive type, and incubation time have significant effects on the adhesion of both species, though interactions were dependent upon species (Table 4). This means that not just one factor was involved in the adhesion process, and that different organisms behave differently depending upon the strains.

Cariogenic streptococci of the same species showed characteristic binding patterns. *S mutans* generally adhered to orthodontic adhesives more than did *S sobrinus*, and there was significant difference in the adhesion according to the strains (Figure 1; Table 1). *S mutans* LM7 showed significantly higher amount of adhesion than did *S mutans* OMZ65, and *S sobrinus* 6715 showed higher amount of adhesion than did *S sobrinus* B13. The order of the amount of adhesion, from greatest to least, was *S mutans* LM7, *S mutans* OMZ65, *S sobrinus* 6715, and *S sobrinus* B13 (Figure 1). This indicates that each strain of the cariogenic streptococci has a characteristic binding pattern.

The amount of the cariogenic streptococci adhesion varied according to the adhesive type (Figure 1). Generally, cariogenic streptococci adhered more significantly to RMGI than to the other four composites, irrespective of the bacterial strain. There were also some differences in adhesion according to the composite type, irrespective of bacterial species, though not statistically significant (Table 4).

Extended incubation time increased the adhesion of cariogenic streptococci. The amount of the bacterial adhesion increased significantly as a result of the extended incubation time, and the amount of adhesion was the highest in the sample after 6 hours of incubation (Table 4).

The saliva coating did not significantly influence the adhesion of the cariogenic streptococci. However, the saliva coating tended to gradually decrease the adhesion compared with the noncoated control (Tables 2 and 3). This decrease in the adhesion amount as a result of saliva coating was evident by the extended incubation time.

There was no interaction effect in *S mutans* strains, whereas interaction effects between strains and incubation times were statistically significant in *S sobrinus* strains ($P < .05$) (Table 4). This is because adhesion to *S sobrinus* 6715 increased more than to *S sobrinus* B13 after 6 hours of incubation (Table 3). This also reflects the characteristic binding pattern of the cariogenic streptococci.

DISCUSSION Return to TOC

A significant difference in the amount of adhesion was observed among the cariogenic streptococci strains (Figure 1; Table 1). *S mutans* LM7 showed significantly higher amount of adhesion than did *S mutans* OMZ65, and *S sobrinus* 6715 showed higher amount of adhesion than did *S sobrinus* B13. This indicates that different strains showed different amounts of adhesion, even though they belong to the same species. The order of adhesion amount was *S mutans* LM7, *S mutans* OMZ65, *S sobrinus* 6715, and *S sobrinus* B13. This suggests

that each strain of cariogenic streptococci has a characteristic adhesion pattern, irrespective of the type of species. However, the adhesion trend of the streptococci was similar among the cariogenic streptococci strains (Figure 1). Irrespective of the adhesive type, *S mutans* LM7 showed the highest amount of adhesion and *S sobrinus* B13 showed the lowest amount of adhesion. In addition, the adhesion trend was not affected by saliva coating and extended incubation time (Table 4).

This study showed that there are substantial differences in the adhesion of cariogenic streptococci to different types of bonding adhesives (Tables 2 and 3). Generally, cariogenic streptococci adhered to RMGI much more than to the composite adhesives (Table 3; Figure 1). The other four composite adhesives also showed some differences but without statistical significance. The differences in the adhesion amount can be explained by the different surface characteristics of each type of adhesive.

Recent work showed that the rough surface of glass ionomer attracted more plaque than did composites[11] and that not all orthodontic adhesives possess identical properties in relation to surface roughness.[12] The increase in the adhesion may be because RMGI has a rougher surface than composite resin. A rough surface increases opportunities for bacterial colonization by increasing the surface area, providing suitable niches for bacterial colonization, and preventing dislodgement of bacterial colonies.[13] Compared with no-mix composites, the mixing procedure for RMGI may partly influence these surface characteristics, as air bubbles formed during mixing can increase surface roughness.

Nevertheless, previous studies have shown in vitro bactericidal or bacteriostatic abilities of the glass ionomer from its surface,[8,9] which differed somewhat from our study. The difference may be attributed to the differences in test microorganisms and methodology. The previous studies used other types of oral bacteria or whole MS as test microorganisms instead of individual cariogenic streptococci. In addition, we did not investigate bacterial growth but investigated only the adhesive capacity of the streptococci. However, other studies have also reported that glass ionomer did not inhibit microbial growth.[11,14]

Saliva coating did not significantly alter the adhesion patterns of cariogenic streptococci, though there were some differences according to the bacterial strain and the adhesive type (Tables 2 and 3). This is consistent with previous studies that showed the salivary coating did not significantly alter the adhesion trend of streptococci to underlying materials.[15,16]

This study showed that fluoride release from the orthodontic adhesive cannot alter the adhesion patterns of cariogenic streptococci. There was no difference in the adhesion amount between fluoride-releasing and non–fluoride-releasing composites. In addition, RMGI increased the adhesion of the cariogenic streptococci significantly more than did the composite adhesives. This can be explained by the fact that the orthodontic bonding adhesive may release fluoride at a rate that affects enamel demineralization rather than bacterial adhesion. Low levels of fluoride may be enough to protect enamel against demineralization but may have little effect on inhibiting growth and adhesion of the cariogenic streptococci. Fluoride from bonding adhesives is delivered to the enamel at the peripheral margin of the bonding adhesive, where it can form the demineralization-resistant fluorapatite on the enamel surface. Several studies have shown that the therapeutic effect of fluoride released in sustained small doses can protect enamel at the periphery of the orthodontic bracket, where most decalcification occurs clinically in orthodontic patients.[17,18] This is also consistent with other work that suggests that there is insufficient fluoride available to inhibit the growth of *S mutans*.[14]

Although RMGI increased the adhesion of cariogenic streptococci in this study, previous studies reported that glass ionomers are significantly more resistant to demineralization than are non–fluoride-releasing composites.[19,20] This may be mainly attributed to the effect of sustained fluoride release from glass ionomers. The resistance to enamel demineralization may also stem from superior marginal adaptation and chemical or physical resistance to the demands of an oral environment. A recent study showed gaps 10 μm in width at the composite-enamel junction around the bracket base, within which bacterial accumulation was constantly detected.[6] The superior physical properties of the glass ionomer can contribute to decrease in the enamel demineralization around the brackets.

This study showed a low amount of cariogenic streptococci adhesion to the orthodontic adhesives, likely because it has an inherently low binding affinity. This is consistent with a previous study that showed that the proportion of *S mutans* was smaller than the other streptococci and comprised only 0.5% of dental plaque after 24 hours.[21] Despite its low binding affinity, the adhesion of cariogenic streptococci may be an important factor in the development of a cariogenic plaque in patients with poor oral hygiene or in caries-active individuals. This is because the microbial mass increases within the first day primarily as a result of cell division.[21]

This study suggests that glass ionomers may not effectively prevent enamel demineralization during long-term orthodontic treatment. The cariogenic streptococci adhered to RMGI significantly more than to the composite adhesives, irrespective of bacterial strains. Fluoride release from the glass ionomer can help protect enamel by forming fluorapatite. However, a glass ionomer can increase the risk of enamel demineralization by increasing adhesion of cariogenic streptococci, which is the primary step for enamel demineralization at the junction between the bonding adhesive and the enamel. Previous studies have shown that the amount of fluoride released from the glass ionomer decreases significantly 1 month after bonding brackets.[22,23] If fluoride-release rates decrease below the critical level for inhibiting enamel demineralization, glass ionomers may therefore be inefficient at preventing enamel demineralization during long-term orthodontic treatment.

CONCLUSIONS Return to TOC

- Each strain of cariogenic streptococci has a characteristic adhesion pattern to the type of orthodontic adhesive.
- The adhesion amount was much higher in the RMGI than in composite adhesives.
- Generally, an extended incubation time increased the level of bacterial adhesion, irrespective of the bacterial strains, whereas the effect of saliva coating did not significantly alter the adhesion trend of cariogenic streptococci.
- The adhesion pattern of the cariogenic streptococci is different between composite adhesives and RMGI, and the adhesion amount is not strongly influenced by fluoride releasing and saliva coating.

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TABLES Return to TOC

Table 1. Orthodontic Bonding Adhesives Investigated in This Study

Bonding Adhesives	Type	
Enlight	Light-cure composite	Ormco/A Compan
Lightbond	Fluoride-releasing light-cure composite	Reliance Orthodor
Monolok2	Light-cure composite	Rocky Mountain C
Transbond XT	Light-cure composite	3M/Unitek, Monrov
Fuji Ortho LC	Fluoride-releasing light-cure resin-modified glass ionomer cement	GC Corporation, T

Table 2. Adhesion Amount of *Streptococcus mutans* LM7 and OMZ65 With Respect to Types of Adhesives, Incubation Times, and Saliva Coating

Strain	Saliva	Incubation Time, h	Adhesives, Mean (SD)			
			Lightbond	Enlight	Monolok2	Transbond XT
LM7	Noncoated	3	0.27 (0.14)	0.24 (0.06)	0.28 (0.13)	0.27 (0.11)
		6	0.35 (0.12)	0.36 (0.10)	0.39 (0.14)	0.44 (0.15)
		Subtotal	0.30 (0.14)	0.29 (0.10)	0.34 (0.14)	0.36 (0.15)
	Saliva coated	3	0.21 (0.10)	0.23 (0.09)	0.25 (0.14)	0.22 (0.08)
		6	0.33 (0.12)	0.32 (0.13)	0.37 (0.16)	0.38 (0.14)
		Subtotal	0.27 (0.13)	0.28 (0.12)	0.30 (0.15)	0.29 (0.13)
	Total	3	0.24 (0.13)	0.24 (0.08)	0.27 (0.14)	0.25 (0.12)
		6	0.34 (0.13)	0.34 (0.12)	0.38 (0.15)	0.41 (0.15)
		Total	0.28 (0.14)	0.28 (0.11)	0.32 (0.15)	0.32 (0.15)
OMZ65	Noncoated	3	0.18 (0.10)	0.22 (0.10)	0.21 (0.14)	0.25 (0.13)
		6	0.33 (0.14)	0.30 (0.10)	0.35 (0.14)	0.43 (0.13)
		Subtotal	0.25 (0.14)	0.26 (0.11)	0.28 (0.14)	0.32 (0.13)
	Saliva coated	3	0.20 (0.11)	0.20 (0.11)	0.25 (0.15)	0.22 (0.07)
		6	0.33 (0.13)	0.30 (0.10)	0.33 (0.19)	0.39 (0.09)
		Subtotal	0.26 (0.12)	0.24 (0.12)	0.29 (0.17)	0.29 (0.12)
	Total	3	0.19 (0.10)	0.21 (0.11)	0.23 (0.14)	0.23 (0.12)
		6	0.33 (0.14)	0.30 (0.10)	0.34 (0.17)	0.41 (0.11)
		Total	0.25 (0.13)	0.25 (0.11)	0.29 (0.16)	0.31 (0.13)

[a] Adhesion amount of cariogenic streptococci was defined as the percentage adhesion.

Table 3. Adhesion Amount of *Streptococcus sobrinus* B13 and 6715 With Respect to Types of Adhesives, Incubation Times, and Saliva Coating