ORIGINAL ARTICLE

Quantitative analysis of adhesion of cariogenic streptococci to orthodontic raw materials

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Introduction: Knowledge of adhesion patterns of cariogenic streptococci to orthodontic materials can provide valuable information on the cause of enamel demineralization during orthodontic treatment. The purpose of this study was to investigate the adhesion of 2 cariogenic streptococci strains to 7 orthodontic raw materials (3 light-cured orthodontic adhesives, 3 bracket raw materials, and hydroxyapatite) with respect to bacterial species, incubation time, and saliva coating. **Methods:** Each material was incubated with unstimulated whole saliva or phosphate-buffered saline solution for 2 hours. Binding assays were then performed by incubating tritium-labeled cariogenic streptococci with each raw material for 3 or 6 hours. **Results:** The degree of adhesion varied by material type. Generally, adhesion of cariogenic streptococci was significantly higher for bonding adhesives than for bracket materials, and adhesion to resin-modified glass ionomer was the highest. A longer incubation time generally increased bacterial adhesion, whereas saliva coating did not significantly influence bacterial adhesion. **Conclusions:** Bonding adhesives around brackets should be removed carefully during the bonding procedure to avoid enamel decalcification. (Am J Orthod Dentofacial Orthop 2008;133:882-8)

The most common side effect of fixed orthodontic treatment is enamel demineralization or white spot formation around orthodontic brackets. The formation of white spots after a fixed orthodontic appliance is placed can occur in up to 50% of patients.[1,2] Clinical observation has indicated that the most common sites for demineralization are peripheral and commonly gingival to the orthodontic bracket.[3,4] Preventing these lesions is an important concern for orthodontists because the lesions are unesthetic, unhealthy, and potentially irreversible.

Enamel demineralization is caused by organic acids produced mainly by cariogenic streptococci,[5] the prime causative organisms of dental caries.[6,7] Of these, *Streptococcus* (*S*) *mutans* and *S sobrinus* have been identified as the main pathogens in dental caries and enamel demineralization.[8] Adhesion and colonization of cariogenic streptococci are considered to play key roles in the development of enamel demineralization related to orthodontic materials, because these materials in the oral cavity present a unique surface that can interact with bacteria, leading to pathogenic plaque formation for enamel demineralization.[9,10] Several studies reported that the placement of fixed orthodontic appliances leads to increases in the volume and number of cariogenic streptococci in dental plaque,[11,12] and the elevated levels of streptococci return to normal after removal of the appliance.[12]

Many factors have been reported to contribute to the development of enamel demineralization. Orthodontic adhesive remaining around the bracket base can be a strong predisposing factor for enamel demineralization, because the rough adhesive surface provides an ideal site for the rapid attachment and growth of oral microorganisms.[13] Orthodontic brackets can also play a role in enamel demineralization, because they provide additional adhesion sites for pathogenic bacteria. However, few studies have investigated the adhesion capacity of cariogenic streptococci to various orthodontic raw materials. Analysis of the adhesion of cariogenic streptococci to orthodontic raw materials will increase our understanding of the factors that cause enamel demineralization.

The purpose of this study was to compare the levels of adhesion of cariogenic streptococci to various orthodontic raw materials to determine which material has a higher retention capacity for streptococci. We used uniformly sized templates of the 7 raw materials.

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Table I. Adhesion of *S mutans* OMZ65 and *S sobrinus* 6715 to orthodontic raw materials (adhesives [fluoride-releasing composite, nonfluoride-releasing composite, and RMGI], bracket raw materials [stainless steel, monocrystalline sapphire, and polycrystalline alumina], and hydroxyapatite), incubation times (3 and 6 hours), and saliva coating (saliva-coated group and noncoated control); adhesion was defined as the percentage of adhesion per cm^2

			Orthodontic raw materials (mean ± SD)		
Strain	*Saliva*	*Incubation time (h)*	*Lightbond**	*Transbond XT†*	*Fuji Ortho LC‡*
S mutans OMZ65	Noncoated	3	0.79 ± 0.44	1.12 ± 0.72	2.15 ± 0.78
		6	1.29 ± 0.71	1.30 ± 0.38	3.20 ± 0.78
		Subtotal	1.04 ± 0.63	1.20 ± 0.59	2.70 ± 0.94
	Saliva coated	3	0.90 ± 0.48	0.96 ± 0.31	2.21 ± 0.83
		6	1.76 ± 0.85	1.87 ± 0.41	2.79 ± 0.98
		Subtotal	1.33 ± 0.80	1.41 ± 0.59	2.50 ± 0.95
	Total	3	0.85 ± 0.45	1.04 ± 0.55	2.18 ± 0.80
		6	1.52 ± 0.82	1.61 ± 0.49	3.00 ± 0.90
		Total	1.18 ± 0.73	1.31 ± 0.59	2.60 ± 0.94
S sobrinus 6715	Noncoated	3	0.55 ± 0.31	0.57 ± 0.32	1.01 ± 0.42
		6	1.01 ± 0.67	0.89 ± 0.39	1.40 ± 0.64
		Subtotal	0.78 ± 0.57	0.70 ± 0.38	1.19 ± 0.56
	Saliva coated	3	0.54 ± 0.42	0.46 ± 0.36	0.86 ± 0.36
		6	0.10 ± 0.68	0.92 ± 0.50	1.72 ± 0.85
		Subtotal	0.77 ± 0.60	0.66 ± 0.47	1.29 ± 0.78
	Total	3	0.55 ± 0.36	0.52 ± 0.34	0.93 ± 0.39
		6	1.00 ± 0.66	0.90 ± 0.44	1.58 ± 0.76
		Total	0.77 ± 0.58	0.68 ± 0.43	1.24 ± 0.68

*Lightbond (Reliance Orthodontics), fluoride-releasing composite.
†Transbond XT (3M Unitek), nonfluoride-releasing composite.
‡RMGI (Fuji-Ortho LC; GC Corporation), RMGI cement.
§Metal: stainless steel (Dae-seung).
¶PCA: polycrystalline alumina (HT Co.).
#MCS: monocrystalline sapphire (HT Co.).

MATERIAL AND METHODS

Seven orthodontic materials (3 light-cured orthodontic adhesives, 3 bracket raw materials, and hydroxyapatite) were included in this study.

The light-cured orthodontic bonding adhesives were a nonfluoride-releasing composite (Transbond XT, 3M Unitek, Monrovia, Calif), a fluoride-releasing composite (Lightbond, Reliance Orthodontics, Itasca, Ill), and a resin-modified glass ionomer cement (RMGI) (Fuji-Ortho LC, GC Corporation, Tokyo, Japan). The specimens were prepared by using fluorine-containing polymer templates with holes 3.0 mm wide and 2.0 mm deep. The templates were positioned on glass slides. Each bonding material was placed into the holes until it was flush with the top of the template. A second slide was placed on top, pushed down to ensure a flat dorsal surface, and then gently removed. All materials were handled according to the manufacturers' instructions and light cured for 40 seconds (20 seconds from the top and 20 seconds from the bottom).

Three bracket raw materials were used: (1) stainless steel (Dae-seung, Seoul, Korea), (2) monocrystalline sapphire (HT Co, Seoul, Korea), and (3) polycrystalline alumina (HT Co.). Each material was provided in a uniform size (4 × 3 × 2 mm) by the manufacturer.

Hydroxyapatite blocks were prepared by the sintering of regent-grade hydroxyapatite powder (Sigma, St Louis, Mo). The powders were pressed uniaxially at 3 GPa by using a hydraulic press (Carber, #3912, Wabash, Ind) to obtain disk-shaped compacts of 3.0 mm in diameter and 2.0 mm in thickness. The compacts were heated at 1200°C for 24 hours in an electric furnace (KT-L-101, Korea Furnace, Seoul, Korea). Crystalline phases were examined by powder x-ray diffraction (Bruker AXS, Karlsruche, Germany).

Unstimulated whole saliva was collected by the spitting method from a healthy 35-year-old volunteer with no acute dental caries or periodontal lesions. Saliva was routinely collected between 7:00 and 9:00 AM to minimize the effects of diurnal variability on salivary composition. The saliva sample was centrifuged at 3500 × g for 5 minutes to remove any cellular debris, and the resulting supernatant was used immediately for the adhesion assays.

The bacterial strains were *S mutans* OMZ65 and *S*

Table I. Continued

Orthodontic raw materials (mean ± SD)				
Metal[§]	*PCA*[¶]	*MCS*[#]	*Hydroxyapatite*	*Subtotal*
0.37 ± 0.17	0.47 ± 0.21	0.61 ± 0.44	1.63 ± 0.68	0.99 ± 0.80
0.51 ± 0.28	0.46 ± 0.20	0.75 ± 0.53	2.11 ± 0.79	1.34 ± 1.09
0.44 ± 0.24	0.47 ± 0.20	0.68 ± 0.49	1.87 ± 0.77	1.17 ± 0.97
0.39 ± 0.30	0.44 ± 0.15	0.56 ± 0.37	1.63 ± 0.83	1.00 ± 0.83
0.53 ± 0.42	0.54 ± 0.34	0.81 ± 0.63	2.28 ± 0.99	1.45 ± 1.10
0.46 ± 0.37	0.49 ± 0.26	0.69 ± 0.53	1.96 ± 0.96	1.22 ± 1.00
0.38 ± 0.24	0.46 ± 0.18	0.58 ± 0.41	1.63 ± 0.75	0.99 ± 0.82
0.52 ± 0.35	0.50 ± 0.28	0.78 ± 0.58	2.20 ± 0.89	1.40 ± 1.10
0.45 ± 0.31	0.48 ± 0.23	0.68 ± 0.51	1.91 ± 0.87	1.19 ± 0.99
0.20 ± 0.11	0.18 ± 0.07	0.28 ± 0.16	0.95 ± 0.48	0.18 ± 0.07
0.28 ± 0.23	0.22 ± 0.10	0.39 ± 0.23	1.38 ± 0.70	0.22 ± 0.10
0.24 ± 0.18	0.20 ± 0.09	0.33 ± 0.20	1.17 ± 0.64	0.20 ± 0.09
0.26 ± 0.25	0.25 ± 0.12	0.33 ± 0.20	0.79 ± 0.39	0.25 ± 0.12
0.28 ± 0.26	0.27 ± 0.13	0.40 ± 0.23	1.44 ± 0.65	0.27 ± 0.13
0.27 ± 0.25	0.26 ± 0.12	0.36 ± 0.21	1.12 ± 0.62	0.26 ± 0.12
0.23 ± 0.20	0.21 ± 0.10	0.30 ± 0.18	0.87 ± 0.44	0.21 ± 0.10
0.28 ± 0.24	0.25 ± 0.12	0.39 ± 0.22	1.40 ± 0.67	0.25 ± 0.12
0.25 ± 0.22	0.23 ± 0.11	0.35 ± 0.21	1.15 ± 0.63	0.23 ± 0.11

sobrinus 6715. Bacteria were stored at −70°C in trypticase (Gibco, Grand Island, NY) with 3% yeast extract broth containing 40% glycerol. Radiolabeling was performed by incubating a loop of bacteria in 10 mL of yeast extract broth containing 50 μ Ci [^{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 mmol/L $NaHCO_3$ (sodium hydrogen carbonate), 1.3 mmol/L $CaCl_2$ (calcium chloride), 0.8 mmol/L $MgCl_2$ (magnesium chloride), and 0.5% bovine serum albumin (HBSS-BSA, pH 7.2). Cell pellets were washed twice and resuspended in HBSS-BSA and adjusted to a final concentration of 5×10^8 cells per mL at A_{660} by using a Petroff-Hauser cell counter (Hauser Scientific Partnership, Horsham, Pa).

Thirty specimens of each material were incubated in 2 mL of unstimulated whole saliva with agitation for 2 hours at room temperature. As a negative control, the same procedure was performed with sterile phosphate-buffered saline solution (pH 7.2) instead of the saliva. The specimens were washed 3 times with phosphate-buffered saline solution and incubated in 2 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 washed 3 times with HBSS-BSA and transferred to scintillation vials. The radiolabeled bacteria were dislodged from the specimens by incubation with 300 μL of 8 mol/L urea, 1 mol/L sodium chloride, and 1% sodium dodecyl sulfate with agitation for 1 hour at 37°C. Then, 3.5 mL of scintillation cocktail was added, and the number of adherent cells was determined by using a liquid scintillation counter (LS-5000TA, Beckman Instruments, Fullerton, Calif). The radioactive counts were divided by total counts per minute of the bacterial suspension solution, and the amount of adhesion was expressed as a percentage of adhesion per unit of area (cm^2). All test samples were counted in triplicate, and each experiment was repeated 6 times. Factorial analysis of variance (ANOVA) was used to analyze the binding affinities and interaction effects of the cariogenic streptococci with respect to species, materials, incubation times,

Table II. Results of 4-way factorial ANOVA for adhesion levels of *S mutans* OMZ65 and *S sobrinus* 6715 to orthodontic raw materials (adhesives [fluoride-releasing composite, nonfluoride-releasing composite, and RMGI], bracket raw materials [stainless steel, monocrystalline sapphire, and polycrystalline alumina], and hydroxyapatite), incubation times (3 and 6 hours), and saliva coating (saliva-coated group and noncoated control)

Source	*df*	*SS*	*MS*	*F*	P	*Multiple comparisons*
Species	1	101.48	101.48	404.23	0.000	*S mutans* > *S sobrinus*
Materials	6	459.41	76.62	305.20	0.000	RMGI > hydroxyapatite > Transbond XT, Lightbond > monocrystalline sapphire > metal, polycrystalline alumina
Incubation times	1	45.80	45.80	182.08	0.000	3 h < 6 h
Saliva coating	1	0.47	0.47	1.86	0.173	
Species × materials	6	44.68	7.45	29.66	0.000	
Species × saliva-coating	1	0.11	0.11	0.45	0.505	
Species × incubation times	1	0.88	0.88	3.45	0.62	
Materials × incubation times	6	23.16	3.86	15.38	0.000	
Materials × saliva coating	6	1.03	0.17	0.69	0.661	
Incubation times × saliva coating	1	0.76	0.76	3.02	0.082	
Error	1426	357.99	0.251			

SS, Sum of squares; *MS*, mean squares.
Multiple comparisons were done by *t* tests with the Bonferroni correction at a significance level of $\alpha = 0.05$.

and saliva coating. Multiple comparisons were performed by *t* tests with the Bonferroni correction at a significance level of $\alpha = 0.05$.

RESULTS

Tables I and II give the results of factorial ANOVA on the adhesion of the cariogenic streptococci with respect to bacterial species, orthodontic raw materials, incubation times, and saliva coating. The results indicate that 3 main factors—orthodontic materials, bacterial species, and incubation time—had significant effects on the adhesion of cariogenic streptococci, but saliva coating did not significantly influence adhesion. There was a statistically significant difference in the interaction effects between materials and species. This indicates that more than 1 factor influences the adhesion process, and that different organisms adhere differently depending on the type of material.

There was a significant difference in the level of adhesion according to the bacterial species (Table I, Fig). Adhesion of *S mutans* OMZ65 to orthodontic materials was significantly greater than that of *S sobrinus* 6715. The adhesion of the cariogenic streptococci was also significantly different according to the type of raw materials (Table I, Fig). Multiple comparisons showed that adhesion of cariogenic streptococci was highest for RMGI and lowest for the metal and polycrystalline alumina materials. In general, adhesion to bonding adhesives was about 2 times higher than adhesion to bracket materials.

Bacterial adhesion was increased significantly by longer incubation time, with the highest adhesion observed for the sample incubated for 6 hours.

Adhesion of cariogenic streptococci to orthodontic raw materials varied according to the bacterial species (Tables I and II). The difference in adhesion between the 2 species was greater for the orthodontic adhesives than for the bracket materials. This was confirmed by a significant interaction effect between species and material ($P < 0.05$, Table II).

DISCUSSION

White spot lesions are associated with enamel demineralization around fixed orthodontic appliances. Orthodontic appliances can play a major role in enamel demineralization because they provide additional surface areas for bacterial adhesion, and their complex design impedes proper access to the tooth surfaces during cleaning. Although several studies have investigated bacterial adhesion to prefabricated brackets, relatively little research has been performed on the raw materials.[14-16] Therefore, limited information is available on which materials are most susceptible to adhesion of cariogenic streptococci. The purpose of this study was to evaluate the level of adhesion of cariogenic streptococci to various orthodontic raw materials.

Our results showed a significant difference in the level of adhesion between the 2 bacterial species (Tables I and II, Fig). In general, adhesion to the materials tested was greater for *S mutans* than for *S sobrinus*. This is consistent with a previous study that reported that *S mutans* adhered more than *S sobrinus* to orthodontic brackets, and that each species of cariogenic streptococci has a characteristic level of adhesion.[14]

We also demonstrated significant differences in adhesion of the cariogenic streptococci according to

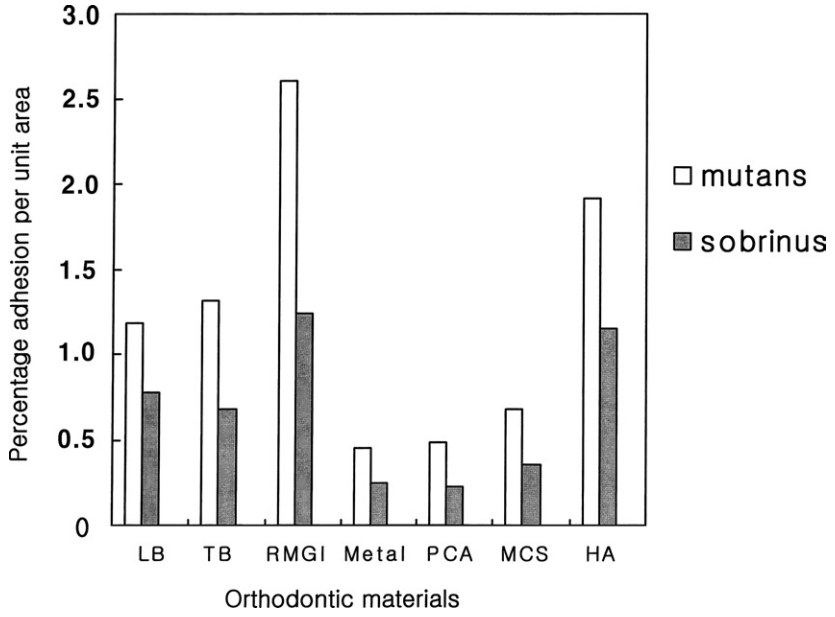

Fig. Total adhesion levels of cariogenic streptococci (*S mutans* OMZ65 and *S sobrinus* 6715) to 7 orthodontic raw materials (LB [Lightbond, Reliance Orthodontics], TB [Transbond XT, 3M Unitek], RMGI [Fuji-Ortho LC; GC Corporation], metal [stainless steel, Korean Smart, Dae-seung], PCA [polycrystalline alumina, Miso II, HT Co.], MCS [monocrystalline sapphire, Miso, HT Co.], and HA [hydroxyapatite block]). The level of adhesion was expressed as the percentage of adhesion per unit of area (cm^2). In general, adhesion to bonding adhesives was significantly higher than to bracket raw materials.

orthodontic material (Tables I and II). In general, the cariogenic streptococci adhered to the bonding adhesives significantly more than to the bracket raw materials. The highest level of adhesion was to RMGI; the lowest level was to metal and polycrystalline alumina bracket materials. The order of adhesion, from highest to lowest, was RMGI, hydroxyapatite, Transbond and Lightbond, monocrystalline sapphire, and metal and polycrystalline alumina.

The high adhesion level for the adhesives can be partly explained by the differences in the surface treatment process. For the bonding adhesives, there was no surface treatment after preparation, whereas the bracket materials were polished and finished as in normal bracket fabrication. These surface-treatment processes made the surface more uniform and regular; this might decrease bacterial adhesion to the bracket materials. In contrast, bonding adhesives can have rough and irregular surfaces that allow bacterial colonization by increasing the surface areas, providing suitable niches for bacterial colonization and preventing the dislodgement of bacterial colonies.[17,18]

We showed that adhesion of cariogenic streptococci to RMGI was higher than to other composite adhesives despite its fluoride-releasing property. There was no significant difference in adhesion between fluoride-releasing and nonfluoride-releasing composites. This suggests that the orthodontic bonding adhesive might release fluoride at a rate that affects enamel demineralization rather than bacterial adhesion; this is consistent with results of a previous study.[19] The high level of adhesion to RMGI might be due to its rough surface, which was shown to attract more plaque than composites.[20] The rough surface increases the surface area and niches, which can provide suitable environments for bacterial adhesion.[17] Compared with no-mix composites, the mixing procedure for RMGI might partly influence surface roughness, because air bubbles formed during mixing can increase surface roughness. In addition, the setting procedure by a specific acid-base reaction between components of RMGI can increase surface-free energy and polarity on the surfaces[21]; these can increase bacterial adhesion according to thermodynamic rules.[17,18] The high adhesion to RMGI indicates that careful attention is needed when using it as a bonding adhesive, even though it releases fluoride.

Eliades et al[22] analyzed surface-free energy of bracket materials and found that ceramic bracket materials have lower surface-free energy than stainless

steel: thus, according to the rules of thermodynamics, stainless steel is a more favorable environment for bacterial adhesion than ceramic.[17,18] In this study, however, the adhesion of cariogenic streptococci was higher for the 2 types of ceramic materials than for stainless steel. The low association between surface-free energy and bacterial adhesion can be explained by the fact that other factors, such as surface roughness, surface charge, and hydrophobicity, can also significantly influence bacterial adhesion.

Nevertheless, previous studies reported that cariogenic streptococci adhered more to stainless steel brackets than to ceramic brackets.[14,15] The difference in adhesion between prefabricated brackets and bracket raw materials might be due to the change in surface properties during the bracket fabrication procedure, since physical and chemical changes in materials can affect relevant surface properties.[14,23] In addition, the adhesion of oral bacteria can be partly influenced by the different base morphology of the prefabricated bracket. For metal brackets, the complex design of the bracket mesh might significantly increase bacterial adhesion.

This study showed that adhesion of cariogenic streptococci to hydroxyapatite was significantly higher than to any orthodontic materials except RMGI (Table I, Fig), although we made the surface of the hydroxyapatite block smoother than bovine incisors (mean surface roughness values of hydroxyapatite blocks and bovine incisors are 0.37 $\pm$ 0.02 μm and 0.99 $\pm$ 0.05 μm, respectively). Although it is difficult to compare a hydroxyapatite block with human enamel, this result suggests that enamel demineralization during orthodontic treatment might not be caused directly by adhesion of cariogenic streptococci to orthodontic materials and stresses the need for careful hygiene control around orthodontic brackets to prevent enamel demineralization.

Saliva coating did not significantly alter the adhesion patterns of cariogenic streptococci in this study. This is consistent with previous studies showing that saliva coating did not significantly alter the adhesion trend of streptococci to the underlying materials.[15,24]

This study showed that orthodontic adhesives have a higher adhesion capacity for cariogenic streptococci than do bracket materials. In addition, orthodontic adhesives are present peripheral to brackets and closer on the enamel surfaces. From a clinical point of view, these characteristics of adhesives are theoretically unfavorable. Furthermore, a recent study reported 10-μm gaps at the adhesive-enamel junction around the bracket base, within which bacterial accumulation was consistently detected.[3] These findings indicate that bonding adhesives around brackets should be removed carefully during the bonding procedure and that rigorous oral hygiene control is required around brackets to decrease the incidence of enamel demineralization.

Although adhesion of cariogenic streptococci to bracket material is lower than that to bonding adhesives, adhesion of cariogenic streptococci to brackets might play a role in the development of cariogenic plaque in patients with poor oral hygiene or in caries-active patients. The remaining bacteria on orthodontic brackets and orthodontic adhesives can grow rapidly on tooth surfaces surrounding the brackets, because microbial mass increases primarily as a result of cell division.[25]

CONCLUSIONS

This study was undertaken to analyze the adhesion of cariogenic streptococci to various orthodontic raw materials. Each species of cariogenic streptococci has a characteristic adhesion pattern with respect to the material type. In general, cariogenic streptococci adhered more to bonding adhesives than to bracket materials. The level of adhesion was highest for RMGI and lowest for metal and polycrystalline alumina bracket materials. A longer incubation time increased the bacterial adhesion irrespective of the species, whereas the effect of saliva coating differed according to material type and bacterial species. Our results underline the importance of rigorous oral hygiene control during the bracket bonding procedure to avoid enamel decalcification around orthodontic appliances.

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