Original Article

Surface Characteristics of Orthodontic Materials and Their Effects on Adhesion of Mutans streptococci

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ABSTRACT

Objective: To test the hypothesis that there are no significant differences in the adhesion of mutans streptococci (MS) to various orthodontic materials based on their surface characteristics.

Materials and Methods: Surface roughness (SR) and surface free energy (SFE) characteristics were investigated for nine different orthodontic materials (four orthodontic adhesives, three bracket raw materials, hydroxyapatite blocks, and bovine incisors) using confocal laser scanning microscopy and sessile drop method. Each material, except the bovine incisors, was incubated with whole saliva or phosphate-buffered saline for 2 hours. Adhesion assays were performed by incubating tritium-labeled MS with each material for 3 or 6 hours.

Results: Orthodontic adhesives had higher SFE characteristics and lower SR than bracket materials. Orthodontic adhesives showed a higher MS retaining capacity than bracket materials, and MS adhesion to resin-modified glass ionomer and hydroxyapatite was highest. Extended incubation time increased MS adhesion, while saliva coating did not significantly influence MS adhesion. SFE, specifically its dispersive and polar components, was positively correlated with MS adhesion, irrespective of saliva coating.

Conclusions: The hypothesis is rejected. This study suggests that SFE characteristics play an important role in the initial MS adhesion to orthodontic materials. (*Angle Orthod.* 2009;79: 353–360.)

KEY WORDS: Surface characteristics; Orthodontic materials; Mutans streptococci; Adhesion

INTRODUCTION

Enamel demineralization around orthodontic brackets is one of the most common side effects during orthodontic treatment using a fixed appliance. The incidence of enamel demineralization can occur in up to 50% of orthodontic patients after using fixed orthodontic appliances.[1,2] Preventing these lesions has been an important concern for orthodontists because the lesions are unesthetic, unhealthy, and potentially irreversible. Enamel demineralization is caused by organic acids produced by mutans streptococci (MS).[3,4] The placement of fixed orthodontic appliances leads to an increase in the level of MS within dental plaque, while MS levels return to normal after removal of the appliance.[5,6]

MS adhesion to various orthodontic materials can play a key role in the pathogenesis of enamel demineralization during orthodontic treatment. The orthodontic adhesive remaining surrounding the brackets can be a risk factor for enamel demineralization because its rough surface provides a site for the rapid attachment and growth of oral microorganisms.[7,8] In addition, orthodontic brackets can play an important role in enamel demineralization, because they provide additional adhesion sites for pathogenic bacteria, and their complex design impedes proper access to the tooth surface for cleaning.

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Table 1. Orthodontic Materials Investigated in This Study

Material Name	Composition	Supplier
Lightbond	Fluoride-releasing light cure composite	Reliance Orthodontics, Itasca, Ill
Transbond XT	Nonfluoride-releasing light cure composite	3M/Unitek, Monrovia, Calif
Transbond Plus	Fluoride-releasing light cure polyacid-modified composite	Reliance Orthodontics, Itasca, Ill
Fuji Ortho LC	Fluoride-releasing light cure resin modified glass ionomer	GC Corporation, Tokyo, Japan
Korean Smart	Stainless steel metal bracket material	Dae-seung, Seoul, Korea
Miso	Monocrystalline sapphire bracket material	HT Co, Seoul, Korea
Miso II	Polycrystalline alumina bracket material	HT Co, Seoul, Korea

Many studies of bacterial adhesion to orthodontic materials have been published.[9–15] It is difficult to compare differences in bacterial adhesion to orthodontic materials in these studies, however, because the size and shape of materials used have varied. If raw materials of uniform size and shape were used, bacterial adhesion studies could more accurately provide an understanding of the accurate risk factors for enamel demineralization. In addition, few studies have explained why oral bacteria differentially adhere to the different orthodontic materials.

Surface characteristics of biomaterials are reported to influence bacterial adhesion in vitro.[16,17] In particular, surface free energy (SFE) and surface roughness (SR) characteristics have a significant impact on this process. Differences in surface characteristics can help explain the differences in MS adhesion to different materials. The purpose of this study was to analyze the initial adhesion of MS to various orthodontic materials in connection with their surface characteristics.

MATERIALS AND METHODS

Hydroxyapatite blocks (HA) were prepared by the sintering of reagent-grade $Ca(PO_4)_3OH$ powder (Sigma, St Louis, Mo). The powders were pressed uniaxially at 3 GPa using a hydraulic press to obtain disk-shaped compacts (3.0-mm diameter and 2.0-mm depth for bacterial adhesion and 12.0-mm diameter and 2.0-mm depth for surface characterization). The compacts were heated at 1100°C for 5 hours in an electric furnace. Crystalline phases were examined by powder x-ray diffractometer (Bruker AXS, Karlsruhe, Germany).

Four light-cure orthodontic adhesives were selected and prepared using Teflon templates (3.0-mm diameter and 2.0-mm depth for bacterial adhesion and 12.0-mm diameter and 2.0-mm depth for surface characterization) according to the manufacturer's instructions (Table 1). Three different kinds of bracket materials were provided in a uniform size ($4.0 \times 3.0 \times 2.0$ mm for bacterial adhesion and $12.0 \times 10.0 \times 2.0$ mm for surface characterization) by their manufacturers (Table 1).

Three freshly extracted, healthy bovine incisors (BI) were cleaned with a rotary brush and pumice, and stored in a 1% aqueous solution of chloramine-T (Junsei Chemical, Tokyo, Japan) at 4°C until the experiments were performed. BI were then embedded individually in an acrylic mold with the labial surface parallel to the mold base. Only BI were used for surface characterization experiments.

The SR was analyzed using confocal laser scanning microscopy (Axiovert 200M, Carl Zeiss, Thornwood, NY). This allows the calculation of the arithmetic mean SR from a mean plane within the sampling area ($245 \times 245 \times 60$ μm). SFE and its component parts, namely the nonpolar (γ^{LW}) and polar acid/base component (γ^{AB}), which is further divided into acid (γ^{+}) and base (γ^{-}) components, were measured by the sessile drop method. Deionized distilled water, 1-bromonaphthalene, and formamide were used as probe liquids. A video camera equipped with an image analyzer (Phoenix 300, Surface Electro Optics, Seoul, Korea) visualized the shape of the drop and determined the contact angle. Right and left contact angles of each drop were averaged, and the total SFE and its components were determined from the averaged contact angles. Each analysis was repeated five times on three specimens of each of the nine materials.

Unstimulated whole saliva (UWS) was collected by the spitting method from a 35-year-old healthy volunteer. Written consent was obtained from the subject, and the research protocol was approved by the institutional review board of the university hospital. The saliva sample was centrifuged at $4500 \times g$ for 5 minutes to remove any cellular debris, and the resulting supernatant was used for adhesion assays.

The bacteria used were *Streptococcus mutans* strain OMZ65 and *Streptococcus sobrinus* strain 6715. 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] thymi-

dine; Amersham Pharmacia Biotech, Piscataway, NJ) for 16 hours anaerobically at 37°C. The tritium-labeled bacteria were harvested by centrifugation at 4500 × *g* for 5 minutes and washed in Hank's Balanced Salt Solution (HBSS; Gibco) supplemented with 4 mM $NaHCO_3$, 1.3 mM $CaCl_2$, and 0.8 mM $MgCl_2$ (HBSS, pH 7.2). Cell pellets were washed twice and resuspended in HBSS and adjusted to a final concentration of 5×10^8 cells per mL at A_{660} using a Petroff-Hauser cell counter (Hauser Scientific Partnership, Horsham, Pa).

Thirty specimens of each material were incubated in 2 mL of UWS with agitation for 2 hours at room temperature. Control specimens were incubated with phosphate-buffered saline (PBS, pH 7.2) under the same conditions. After washing three times in PBS, saliva-coated samples were incubated with 1×10^9 tritium-labeled bacteria in 2 mL of HBSS containing 0.5% bovine serum albumin (HBSS) under agitation for either 3 or 6 hours at 37°C. Noncoated controls were incubated with HBSS containing 1×10^9 tritium-labeled bacteria under the same conditions. All specimens were then washed three times with HBSS-BSA and transferred to scintillation vials. The radiolabeled bacteria were dislodged using 300 μL of 8 M urea, 1 M NaCl, and 1% sodium dodecyl sulfate under agitation for 1 hour at 37°C. Then, 3.5 mL of scintillation cocktail was added, and the number of adherent cells was determined using 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 adhesion was expressed as percentage adhesion per unit area (cm^2). All test samples were counted in triplicate and each experiment was repeated six times.

The differences in surface characteristics were analyzed using one-way analysis of variance (ANOVA). A four-way ANOVA was used to analyze MS adhesion with respect to bacterial strain, adhesive type, incubation time, and saliva coating. Multiple comparisons were done with *t*-tests using the Bonferroni correction. Spearman rank correlation coefficients were calculated to analyze the relationship between surface characteristics of the raw materials and MS adhesion. All values were considered significant at $P < .05$.

RESULTS

There were significant differences in SR between materials (Table 2). The monocrystalline sapphire (MCS) bracket material had the smoothest surfaces, while BI had the roughest surfaces.

Significant differences in SFE and its components were found between materials (Table 2). HA had the highest SFE, γ^{AB}, and γ^-, while bracket metal had the lowest SFE, γ^{LW}, and γ^{AB}. Generally, orthodontic adhesives had higher SFE, γ^{LW}, and γ^{AB} than bracket materials. Among bracket materials, stainless steel metal showed lower SFE and γ^{AB} than polycrystalline alumina (PCA) and MCS. Among the adhesives, resin-modified glass ionomer (RMGI) and compomer had higher SFE, γ^{LW}, and γ^{AB} than the composite adhesives. The SFE characteristics of compomer were intermediate between the RMGI and composites, but closer to those of RMGI than those of composites.

There was a significant difference in the MS adhesion according to the bacterial species (Tables 3 and 4). Adhesion of *S. mutans* OMZ65 to orthodontic materials was significantly greater than that of *S. sobrinus* 6715. The MS adhesion was also significantly different according to the types of raw materials (Table 3). Multiple comparisons demonstrated that MS adhesion was highest for RMGI and HA, and lowest for bracket materials. In general, adhesion to adhesives was significantly higher than adhesion to bracket materials. Saliva coating did not significantly influence MS adhesion, and there was no significant difference in MS adhesion between saliva coated samples and noncoated control. Bacterial adhesion was increased by extended incubation time, with the highest adhesion observed for the samples receiving 6-hour incubation (Table 3).

MS adhesion to orthodontic materials varied according to the incubation time (Table 3). The difference in adhesion between 3- and 6-hour incubations was greater for orthodontic adhesives and HA than for bracket materials. This was confirmed by a significant interaction effect between material and incubation time ($P < .05$) (Table 4).

The Spearman rank correlation test showed that MS adhesion was positively correlated with SFE, γ^{LW}, and γ^{AB} irrespective of bacterial species and saliva-coating (Table 5). Not significantly related to MS adhesion were γ^+ and γ^-.

DISCUSSION

This study demonstrated significant differences in MS adhesion to various orthodontic materials (Table 3). In general, MS adhered to orthodontic adhesives significantly more than to bracket materials. RMGI and hydroxyapatite showed the highest MS adhesion, while the three bracket materials showed the lowest MS adhesion. However, there were no significant differences in MS adhesion between the three bracket materials. The order of adhesion, from highest to lowest, was: RMGI and hydroxyapatite, compomer, Transbond XT and Lightbond, and bracket materials. The difference in the adhesion amount can be ex-

Table 2. One-Way Analysis of Variance (ANOVA) Results for Surface Roughness, Surface Free Energy, and Surface Free Energy Components (Dispersive, Polar, Acid, and Base Components) in Various Orthodontic Raw Materials*

	Orthodontic Raw Materials*			
	Adhesives			
	LB[1]	TB[2]	Compomer[3]	RMGI[4]
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Surface roughness, μm	0.43 (0.01)[d]	0.38 (0.02)[c]	0.42 (0.01)[d]	0.39 (0.02)[d]
Surface free energy, mJ/m	41.49 (1.09)[c]	41.73 (1.57)[c]	46.77 (0.96)[d]	48.28 (0.85)[e]
Dispersive component, mJ/m	40.08 (0.97)[d]	40.80 (0.85)[de]	42.11 (0.73)[f]	42.21 (0.74)[f]
Polar component, mJ/m	1.46 (1.09)[c]	0.90 (1.39)[bc]	4.69 (0.80)[e]	5.98 (0.71)[f]
Acid component, mJ/m	0.11 (0.10)[a]	0.07 (0.12)[a]	0.88 (0.33)[b]	2.28 (0.67)[e]
Base component, mJ/m	8.39 (2.78)[c]	8.71 (1.51)[c]	6.44 (1.48)[b]	4.20 (1.03)[a]

* Measurements with the same superscript letter indicate no statistically significant difference using multiple comparisons with the Bonferroni correction at a significance level of $p < .05$.

[1] LB (Lightbond): Fluoride-releasing composite.
[2] TB (Transbond XT): Nonfluoride-releasing composite.
[3] Compomer (Transbond Plus): Polyacid-modified composites.
[4] RMGI: Resin-modified glass ionomer cement.
[5] Metal: Stainless steel metal.
[6] PCA: Polycrystalline alumina.
[7] MCS: Monocrystalline sapphire.
[8] HA: Hydroxyapatite block.
[9] BI: Bovine incisor.

Table 3. Adhesion[a] of *Streptococcus mutans* OMZ65 and *Streptococcus sobrinus* 6715 to Eight Orthodontic Raw Materials, Incubation Times (3 and 6 Hours), and Saliva-Coating (Saliva-Coated Group and Noncoated Control)

			Orthodontic Raw Materials			
			Orthodontic Adhesives			
			LB[1]	TB[2]	Compomer[3]	RMGI[4]
Strain	Saliva	Incubation Time, Hours	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Streptococcus mutans OMZ65	Noncoated	3	0.64 (0.38)	0.78 (0.47)	1.51 (0.56)	1.85 (0.96)
		6	1.00 (0.50)	1.01 (0.31)	1.58 (0.42)	2.26 (0.51)
		Subtotal	0.82 (0.46)	0.90 (0.40)	1.55 (0.49)	2.06 (0.77)
	Saliva-coated	3	0.71 (0.48)	0.80 (0.36)	1.28 (0.50)	1.82 (0.67)
		6	1.28 (0.53)	1.40 (0.27)	1.41 (0.54)	2.23 (0.79)
		Subtotal	1.00 (0.57)	1.09 (0.43)	1.34 (0.52)	2.02 (0.74)
	Total	3	0.68 (0.42)	0.79 (0.40)	1.38 (0.53)	1.84 (0.81)
		6	1.14 (0.52)	1.21 (0.34)	1.48 (0.49)	2.25 (0.65)
		Total	0.91 (0.52)	1.00 (0.42)	1.43 (0.51)	2.05 (0.75)
Streptococcus sobrinus 6715	Noncoated	3	0.65 (0.41)	0.75 (0.62)	1.12 (0.27)	1.61 (0.60)
		6	0.87 (0.56)	0.95 (0.67)	1.52 (0.15)	1.95 (0.77)
		Subtotal	0.76 (0.49)	0.85 (0.63)	1.32 (0.30)	1.76 (0.77)
	Saliva-coated	3	0.49 (0.37)	0.65 (0.48)	1.14 (0.10)	1.38 (0.55)
		6	0.73 (0.47)	0.99 (0.73)	1.34 (0.16)	1.99 (0.59)
		Subtotal	0.61 (0.42)	0.82 (0.63)	1.24 (0.16)	1.67 (0.63)
	Total	3	0.58 (0.39)	0.70 (0.55)	1.13 (0.20)	1.49 (0.57)
		6	0.81 (0.51)	0.97 (0.68)	1.43 (0.18)	1.97 (0.65)
		Total	0.70 (0.46)	0.84 (0.62)	1.28 (0.24)	1.71 (0.65)

[a] Adhesion was defined as the percentage adhesion per cm^2.
[1] Lightbond: Fluoride-releasing composite.
[2] Transbond XT: Nonfluoride-releasing composite.
[3] Compomer: Polyacid-modified composites.
[4] RMGI: Resin-modified glass ionomer cement.
[5] Metal: Stainless steel metal.
[6] PCA: Polycrystalline alumina.
[7] MCS: Monocrystalline sapphire.
[8] HA: Hydroxyapatite block.
[9] Multiple comparisons were done by the Bonferroni correction at a significance level of $p < .05$.

Table 2. Extended.

Orthodontic Raw Materials*				
Bracket Materials			Others	
Metal[5]	PCA[6]	MCS[7]	HA[8]	BI[9]
Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
0.62 (0.03)[e]	0.76 (0.03)[f]	0.26 (0.03)[a]	0.35 (0.09)[b]	0.99 (0.03)[g]
32.45 (1.30)[a]	39.79 (2.82)[c]	35.33 (2.67)[b]	52.22 (1.06)[f]	40.67 (1.45)[c]
34.56 (0.76)[a]	37.08 (0.83)[b]	35.88 (1.89)[a]	41.24 (0.90)[e]	39.04 (1.07)[c]
−2.04 (1.11)[a]	2.71 (1.69)[d]	0.31 (1.35)[b]	11.04 (1.46)[g]	1.63 (0.85)[cd]
0.19 (0.15)[a]	1.37 (1.09)[c]	0.24 (0.38)[a]	1.85 (0.57)[d]	0.26 (0.24)[a]
5.98 (2.10)[b]	3.14 (1.65)[a]	3.34 (2.75)[a]	18.08 (5.68)[d]	4.62 (2.19)[ab]

Table 3. Extended.

Orthodontic Raw Materials				
Bracket Materials			Hydroxyapatite	
Metal[5]	PCA[6]	MCS[7]	HA[8]	Multiple Comparisons[9]
Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
0.37 (0.15)	0.47 (0.19)	0.61 (0.44)	1.63 (0.64)	
0.51 (0.25)	0.46 (0.18)	0.75 (0.53)	2.11 (0.77)	*S. mutans* >
0.44 (0.22)	0.47 (0.17)	0.68 (0.46)	1.87 (0.73)	*S. sobrinus*
0.39 (0.28)	0.45 (0.13)	0.56 (0.34)	1.63 (0.74)	
0.53 (0.42)	0.54 (0.29)	0.81 (0.58)	2.28 (0.97)	RMGI, HA >
0.46 (0.36)	0.49 (0.23)	0.69 (0.47)	1.96 (0.90)	compomer >
0.38 (0.22)	0.46 (0.16)	0.58 (0.38)	1.63 (0.67)	TB, LB >
0.52 (0.34)	0.50 (0.24)	0.78 (0.54)	2.20 (0.86)	MCS, Metal, PCA
0.45 (0.31)	0.49 (0.20)	0.68 (0.47)	1.91 (0.81)	
0.20 (0.11)	0.17 (0.06)	0.28 (0.14)	1.14 (0.42)	
0.28 (0.23)	0.23 (0.09)	0.39 (0.21)	1.78 (0.43)	
0.24 (0.18)	0.20 (0.08)	0.33 (0.18)	1.47 (0.53)	
0.26 (0.25)	0.24 (0.12)	0.33 (0.20)	0.99 (0.34)	
0.28 (0.26)	0.28 (0.13)	0.40 (0.23)	1.73 (0.52)	
0.27 (0.25)	0.26 (0.12)	0.36 (0.19)	1.39 (0.58)	
0.23 (0.20)	0.21 (0.10)	0.30 (0.17)	1.07 (0.38)	
0.28 (0.24)	0.26 (0.11)	0.39 (0.20)	1.76 (0.47)	
0.25 (0.22)	0.23 (0.11)	0.35 (0.19)	1.43 (0.54)	

Table 4. Results of Four-Way Factorial Analysis of Variance (ANOVA) for Adhesion Levels of *Streptococcus mutans* OMZ65 and *Streptococcus sobrinus* 6715 to Various Orthodontic Raw Materials, Incubation Times (3 and 6 Hours), and Saliva-Coating (Saliva-Coated Group and Noncoated Control)

Source	DF[a]	SS[b]	MS[c]	F	*P*	Multiple Comparisons[d]
Species	1	10.16	10.16	47.44	.000	*S. mutans* > *S. sobrinus*
Materials	7	190.81	27.26	127.27	.000	Resin modified glass ionomer, hydroxyapatite > compomer > Transbond XT, Lightbond > monocrystalline sapphire, metal, polycrystalline alumina
Incubation times	1	11.20	11.20	52.30	.000	3 hours < 6 hours
Saliva-coating	1	0.00	0.00	0.02	.890	
Species × materials	7	1.69	0.24	1.12	.346	
Species × saliva-coating	1	0.16	0.16	0.76	.383	
Species × incubation times	1	0.01	0.01	0.06	.806	
Materials × incubation times	7	5.13	0.73	3.42	.001	
Materials × saliva-coating	7	0.70	0.10	0.48	.853	
Incubation times × saliva-coating	1	0.19	0.19	0.89	.345	

[a] DF indicates degree of freedom.
[b] SS indicates sum of squares.
[c] MS indicates mean squares.
[d] Multiple comparisons were done by the Bonferroni correction at a significance level of $p < .05$.

plained by the difference in the surface characteristics of each material.

The present study showed significant differences in MS adhesion among bracket materials, which is inconsistent with the results of a previous study[14] that showed that there were no significant differences in adhesion of *S. mutans* to different types of brackets. This may be mainly due to the differences in materials used. We used raw bracket materials with the same size and shape, but the previous study used commercial brackets. The differences in MS adhesion may be partly explained by the differences in strains used and culture condition.

Two surface characteristics influence the adhesion amount of bacteria to orthodontic material surfaces: SR and SFE.[17] A rough surface provides opportunities for bacterial adhesion by increasing the surface area and providing suitable niches. Differences in SR were found among the materials. Bracket materials showed higher SR than adhesives except MCS. The highest SR was shown in the bovine incisors. However, no significant relationship was found between SR and MS adhesion (Table 5). This can be partly explained by the relatively minor differences in SR (less than 0.5 μm) among the materials despite statistical significance, which is consistent with previous studies reported that minor variations in SR have no significant effect on bacterial adhesion or on the contact angles for SFE.[17,18]

A material with high SFE will attract more bacteria to its surface than one with low SFE according to thermodynamic rule.[17] In particular, nonspecific physicochemical interactions, such as van der Waals interactions and acid-base interactions, play an important role in initial bacterial adhesion and can be defined by SFE and its components.[18] There were significant differences in SFE and its components among the orthodontic materials. This study showed that orthodontic adhesives, particularly, RMGI and compomer, had significantly higher SFE, γ^{LW}, and γ^{AB} than bracket materials. These differences can be mainly due to their different physicochemical compositions. Partly, these differences in SFE characteristics can be explained by the differences in surface reactivities among them. Bracket materials may have less reactive surfaces than orthodontic adhesives, because bracket materials are made from stable metal alloy or ceramics in nature and have stable crystal structure and orientation. In case of RMGIs and compomers containing highly reactive fluoro-aluminosilicate glass fillers, however, polymerization and polyacid neutralization including fluoride release are continued in the aqueous phase for long periods.[19] In addition, relatively impermeable composites are reported to react with exogenous components such as fluoride after exposure.[20]

Eliades et al[21] analyzed surface energy of bracket materials and found that ceramic bracket materials have lower surface free energy than stainless steel metal. However, the present study showed that SFE of bracket metals was lower than that of ceramic brackets. This may be due to the different materials used. We have measured SFE from various bracket metals, but bracket metals showed significantly different SFE characteristics according to manufacturers (data not shown). The differences may be mainly due to the differences in the type of metal investigated, which can influence bacterial binding. The differences in the fabrication procedures may partly influence the differences in SFE components, since physical and

Table 5. Spearman Rank Correlation Coefficients for Surface Characteristics and Bacterial Adhesion

Strain	Saliva	Incubation Time, Hours	Surface Roughness	Surface Free Energy	Dispersive Component	Polar Component	Acid Component	Base Component
Streptococcus mutans OMZ65	Noncoated	3	−0.50	0.952***	0.905**	0.810*	0.500	0.452
		6	−0.52	0.905**	0.857**	0.714*	0.429	0.524
	Saliva-coated	3	−0.50	0.952***	0.905**	0.810*	0.500	0.452
		6	−0.54	0.976***	0.881**	0.833**	0.476	0.571
Streptococcus sobrinus 6715	Noncoated	3	−0.52	0.905**	0.857**	0.714*	0.429	0.524
		6	−0.52	0.905**	0.857**	0.714*	0.429	0.524
	Saliva-coated	3	−0.45	0.857**	0.905**	0.667	0.381	0.452
		6	−0.51	0.934**	0.886**	0.776*	0.467	0.491
Total	Noncoated	3	−0.50	0.952***	0.905**	0.810*	0.500	0.452
		6	−0.53	0.905**	0.857**	0.714**	0.429	0.524
	Saliva-coated	3	−0.50	0.952***	0.905**	0.810*	0.500	0.452
		6	−0.50	0.952***	0.905**	0.810*	0.500	0.452

* $P < .01$; ** $P < .001$; *** $P < .0001$.

chemical changes in materials can affect relevant surface properties.[22]

The high SFE of the RMGI and HA, and the low SFE of stainless steel metal explains the finding that RMGI and HA had the higher MS adhesion, while stainless steel had lower MS adhesion than other materials (Table 3). This is confirmed by the significant positive correlation between SFE and MS adhesion (Table 5). In particular, the Spearman rank correlation test showed that γ^{LW} and γ^{AB} were significant related to MS adhesion. This can be explained by the fact that a higher polarity will create strong initial bacterial adhesion, because polar interactions are one of the important mechanisms in the initial stage of bacterial adhesion.[23] In addition, the dispersive component associated with van der Waals interactions plays an important role in initial bacterial adhesion.[24] Therefore, greater MS adhesion to orthodontic adhesives, particularly RMGI and compomer, than to bracket materials can also be explained by the higher SFE, γ^{LW}, and γ^{AB}.

Previous studies reported that saliva-coating influences bacterial adhesion by changing the SFE of the underlying materials[17] or by mediating bacterial adhesion by acting as a specific binding receptor.[25] In this study, however, saliva-coating did not significantly alter MS adhesion to orthodontic materials (Tables 3 and 4). In addition, MS adhesion was significantly related to SFE, γ^{LW}, and γ^{AB} after saliva-coating. This suggests that saliva-coating may not significantly alter the surface characteristics of the underlying materials.[26,27] This may be partly due to the fact that salivary proteins formed on the surface do not significantly mediate the initial adhesion of MS.[11]

BI and HA were used as primary substitutes of human enamel to simulate surface characteristics due to the difficulty in achieving an unpolished flat and large enamel surface. However, this study showed that the surface characteristics of HA are significantly different from those of BI. HA had a smoother surface, while BI showed significantly lower SFE, γ^{LW}, γ^{AB}, γ^{+} and γ^{-}. HA had the highest SFE, γ^{LW}, and γ^{AB}, which may explain its high rates of MS adhesion. In contrast to HA, the surface characteristics of BI are closer to those of composite adhesives than other materials (Table 2). Considering that SR is not a significant factor for MS adhesion and bovine enamel is a reliable counterpart of human enamel in terms of SFE characteristics,[28] MS adhesion to human enamel may be similar to MS adhesion to composite adhesives and be greater than MS adhesion to bracket materials.

This study showed that orthodontic adhesives may have a higher MS-retaining capacity than bracket materials. In addition, when used in patients, orthodontic adhesives are located closer to the enamel surfaces than are the brackets. From a clinical point of view, these characteristics are favorable for enamel demineralization. These findings indicate that orthodontic adhesives could pose a more serious risk of enamel demineralization than the risks posed by brackets. These results suggest that orthodontic adhesives around brackets should be removed carefully during the bonding procedure and that rigorous oral hygiene control around adhesives should be required to decrease the incidence of enamel demineralization during orthodontic treatment.

CONCLUSIONS

- Orthodontic adhesives have a higher MS-retaining capacity than bracket materials.
- The effect of surface characteristics on the MS adhesion was not significantly influenced by a saliva coating.
- There was a significant correlation between SFE components and MS adhesion.
- Higher SFE, specifically dispersive and polar com-

ponents, had a favorable effect on MS adhesion to orthodontic materials.

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