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ORIGINAL ARTICLE

The effects of orthodontic bonding steps on biofilm formation of *Streptococcus mutans* in the presence of saliva

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Abstract

Objective. To investigate the effects of various orthodontic bonding steps on biofilm formation of *Streptococcus mutans* in the presence of saliva. ***Materials and methods:*** Hydroxyapatite (HA) and orthodontic adhesive (AD) disks were prepared to a uniform size. HA disks were etched with 37% phosphoric acid gel in the etched group (HE). In the primed group (HP), Transbond XT primer was applied to the etched HA surface and light-cured. For biofilm formation, *Streptococcus mutans* was grown on each specimen in a biofilm medium with either glucose or sucrose in the presence of fluid-phase UWS (F-UWS) or surface adsorbed saliva (S-UWS). The adherent bacteria were quantified by enumeration of the total viable counts of bacteria. Biofilms formed on each surface were examined by scanning electron microscopy. ***Results.*** When glucose was used, both F-UWS and S-UWS suppressed biofilm formation of *S. mutans.* Compared to HA and HE, biofilm formation was significantly inhibited on HP and AD in the presence of glucose. Biofilm-forming patterns that were inhibited by saliva were restored in a sucrose-containing medium. F-UWS promoted biofilm formation on HA and HE, while S-UWS significantly promoted biofilm formation on HP. *S. mutans* developed biofilm better on HA and HE than on AD when sucrose was used as the sole carbohydrate source. ***Conclusions.*** This study suggests that the biofilm development by *S. mutans* is significantly influenced by the orthodontic bonding procedure. Biofilm formation of *S. mutans* was inhibited on AD more than other surfaces, irrespective of the presence of saliva or a carbohydrate source.

Key Words: *orthodontic bonding, biofilm formation, Streptococcus mutans, carbohydrate*

Introduction

Fixed orthodontic treatments are associated with extensive plaque accumulation which induces gingival inflammation around orthodontic appliances. Enamel demineralization, appearing as a white spot around orthodontic appliances, is the most common side-effect of fixed orthodontic treatments [1,2]. The incidence of white spot formation after using a fixed orthodontic appliance can occur in up to 50% of patients [3,4]. Preventing these lesions has been an important focus for orthodontists because the lesions are unaesthetic and potentially irreversible.

Streptococcus mutans is a primary agent for enamel demineralization, because this species is an important component of dental biofilms which are associated with enamel demineralization. Adhesion and biofilm formation of *S. mutans* is mediated by sucrose-dependent and sucrose-independent mechanisms [5]. Sucrose-independent adherence is significantly influenced by the presence of saliva, because several surface adhesions around cell surfaces can bind to salivary components formed on teeth [6]. On the contrary, sucrose-dependent adherence is mediated by glucan binding proteins and water-insoluble glucans produced from sucrose by glucosyltransferase enzymes (GTFs) of *S. mutans* [7].

Orthodontic bonding has proved to be very successful and has led to dramatic changes in the field of orthodontics. The bonding steps include various procedures, such as etching, priming and the application of orthodontic adhesive before bonding the brackets. The primary effect of etching is to increase the surface area and thereby change the surface from a low

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energy, hydrophobic surface to a high energy, hydrophilic surface. Priming is needed to achieve proper bond strength and protect the enamel after acid-etching [8]. Many studies have been performed to improve the orthodontic bonding system. However, few studies have investigated the biological activities of *S. mutans* on the tooth surfaces after various surface treatments for bracket bonding. Changes in tooth surfaces followed by the bonding procedure may not only affect bond strength, but also significantly influence biofilm development by these organisms. The purpose of this study was to analyze the effects of various orthodontic bonding procedures on biofilm development by *S. mutans* in the presence of saliva using two carbohydrate sources.

Materials and methods

Disk preparation

Hydroxyapatite disks were prepared to a uniform size (10.0-mm diameter and 2.0-mm thickness) by the sintering of regent-grade (>95%) $Ca(PO_4)_3OH$ powder (Aldrich, St Louis, MO, USA) as previously described [9]. The disks were divided into three groups according to the type of surface treatment; no surface treatment control (HA), acid-etched (HE) and primed (HP). In the HE group, the hydroxyapatite disk was etched with 37% phosphoric acid gel, rinsed with deionized water and air-dried. Transbond XT primer (3M, Monrovia, CA, USA) was applied to the etched surface in a thin film and light-cured for 20 s with an Ortholux LED (3M) in the HP group. For the adhesive (AD) group, Transbond XT composite adhesive (3M) disks were prepared to the same size as the HA group using Teflon templates as previously described [9]. The adhesive disks were light-cured for 20 s each from the top and bottom according to the manufacturer's instruction.

Saliva collection

Unstimulated whole saliva (UWS) was collected by the spitting method from healthy volunteers as previously described [9]. The saliva sample was centrifuged at 3500 × *g* for 10 min to remove any cellular debris and the resulting supernatant was used after filter-sterilization through a Stericup & Steritop (Millipore, Billerica, MA, USA).

Biofilm formation

S. mutans serotype c UA159 was used in this study. Overnight cultures of *S. mutans* were transferred to a pre-warmed brain heart infusion (BHI) broth and grown at 37°C in a 5% CO_2 aerobic atmosphere to its late exponential phase (OD_{600} = 0.5, ~ 6.5 × 10^7 colony forming unit/ml). The cultures were then diluted 1:100 in a pre-warmed biofilm medium (BM) with either 20 mM glucose (BM-glucose) or 20 mM sucrose (BM-sucrose) as a carbohydrate source, as previously described [10]. Biofilm assays were performed using three different UWS treatments: fluid-phase UWS (F-UWS), surface-adsorbed UWS (S-UWS) and no UWS treatment (control). For the experiments with S-UWS, each disk was conditioned with 1.0 mL of UWS in the well at 37°C for 2 h with gentle shaking, followed by three washes with PBS. After air drying for 30 min, 2.0 mL of the diluted cell cultures was inoculated into the well containing the disk. For experiments with F-UWS, 2.0 mL of the diluted cell cultures was inoculated into the well containing a disk concurrently with 200 μL of UWS. Two milliliters of cell suspension was inoculated to wells containing a disk without any UWS treatment for control.

Biofilm evaluation

Biofilms were allowed to form by incubating at 37°C in a 5% CO_2 for 24 h. The culture medium was then decanted and the disks were washed twice with 1.0 mL sterile PBS to remove planktonic and loosely bound cells. Each disk was transferred to a conical tube containing 3 mL PBS. The adherent bacteria were detached by sonication using four 30-s pulses at 25 W with three 30-s intermittent cooling stages in a chilled ice box. The cell suspensions were serially diluted, plated on BHI agar and incubated at 37°C for 2 days before the colonies were counted. Colony counts were expressed as a colony forming unit per unit area (cm^2). All assays were carried out only using new disks and performed in duplicate and repeated five times.

For biofilms that were to be examined by scanning electron microscopy (SEM), the biofilms were grown as described above and analyzed with a magnification set at 3000× using a S-4700 microscope (Hitachi, Tokyo, Japan).

Surface roughness measurement

The arithmetic mean surface roughness (SR) of each specimen was analyzed within the sampling area (245 × 245 × 60 μm) using confocal laser scanning microscopy (Axiovert 200M, Carl Zeiss, Thornwood, NY, USA). Each SR reading was performed 3-times on three different areas for each of the three specimens.

Statistical analysis

Factorial analysis of variance (ANOVA) was used to analyze the amount of biofilm cells with respect to the surface type and UWS treatment. SR was analyzed using one-way ANOVA. Multiple comparisons were

performed by *t*-tests using the Bonferroni correction to compare differences between the groups. All values were considered significant when $p < 0.05$.

Results

When glucose was used as a sole carbohydrate source, both F-UWS and S-UWS significantly suppressed biofilm development by *S. mutans*, compared to the no-UWS treatment group (Table I). In addition, there were differences between F-UWS and S-UWS. F-UWS resulted in greater inhibition of biofilm formation than S-UWS (no UWS treatment > S-UWS > F-UWA). The inhibition of biofilm development was not due to the alterations in bacterial growth by UWS, because there was no significant difference in bacterial growth rates in the presence of UWS (data not shown). These findings indicate that both F-UWS and S-UWS have a specific capacity to hinder biofilm development. There were also significant differences in biofilm development by *S. mutans* according to the surface type. Compared to HA and HE, biofilm formation was significantly inhibited on HP and AD in BM-glucose.

Biofilm formation patterns in BM-sucrose were significantly different from those in BM-glucose (Table II). Both UWS treatments did not inhibit biofilm development in BM-sucrose. In addition, F-UWS or S-UWS uniquely influenced biofilm-forming capacities of *S. mutans* according to the surface type. F-UWS significantly promoted biofilm development on HA and HE, while S-UWS significantly promoted biofilm development on HP. However, both UWS treatments did not significantly influence biofilm formation of *S. mutans* on the AD surfaces. Irrespective of UWS treatments, there was the least amount of biofilm development on AD in BM-sucrose.

Biofilms observed by SEM were consistent with the quantitative biofilm assays. Biofilm development was significantly inhibited by both UWS treatments in BM-glucose. Adherent cell clusters were shown in the absence of both UWS treatments (Figures 1A–D) relative to the short scattered chains and microcolonies on the same surfaces in the presence of S-UWS (Figures 1E–H) or F-UWS (Figures 1I–L). There were also significant differences according to the surface type. Adherent cell clusters and microcolonies were more frequently found on HA (Figures 1A, E and I) and HE (Figure 1B, F and J) than on HP (Figures 1C, G and K) and AD (Figures 1D, H and L), although cell clusters were more scattered in the presence of F-UWS or S-UWS. In BM-sucrose, *S. mutans* formed better biofilms than they did in BM-glucose (Figure 2). Thick cell aggregates were found in abundant polysaccharide matrices, which made it difficult to observe evident differences in microscopic characteristics among the specimens. However, cell aggregates were less frequently found on AD than the other surfaces (Figures 2D, H and L).

There was a significant difference in SR among the specimens (Table III). HE showed the roughest surface, while AD exhibited the smoothest surface (AD < HA, HP < HE).

Discussion

Dental plaque, an oral biofilm that is formed around orthodontic brackets, plays an important role in the etiology of enamel demineralization. Although the oral flora is quite diverse and complex, *S. mutans* has generally been regarded as the primary etiologic agent of enamel demineralization and dental caries in humans. This species adheres and accumulates on the tooth surface by generating extracellular polysaccharides from sucrose in the oral cavity. These particular abilities of *S. mutans* are essential for the formation and structural integrity of the biofilm. Thus, the control of *S. mutans* biofilm is one of the targets for the prevention of enamel demineralization during orthodontic treatment and knowledge on biofilm formation of *S. mutans* is essential to develop a promising approach for the prevention of dental caries.

Biofilm development by *S. mutans* was significantly inhibited by both F-UWS and S-UWS in BM-glucose, regardless of the surface type (Table I and Figure 1). However, S-UWS resulted in less inhibition of biofilm formation than was observed

Table I. Biofilm development by *Streptococcus mutans* UA159 on various surfaces in biofilm medium supplemented with 20 mM glucose. The amounts of biofilms were expressed as a colony forming unit (CFU) per unit area (cm^2).

	Surface type ($\times 10^9$ CFU/cm^2)				
Saliva treatment	HA ($M \pm$ SD)	HE ($M \pm$ SD)	HP ($M \pm$ SD)	AD ($M \pm$ SD)	Significance*
No treatment	5.34 ± 2.52	5.16 ± 1.85	1.77 ± 1.61	2.42 ± 1.04	HA, HE > HP, AD Control > surface-adsorbed > fluid-phase
Surface-adsorbed	3.31 ± 1.07	2.75 ± 0.58	1.56 ± 1.22	1.62 ± 0.78	
Fluid-phase	0.76 ± 0.19	1.30 ± 0.32	0.48 ± 0.42	0.59 ± 0.43	

HA, Hydroxyapatite disk, no surface treatment; HE, Hydroxyapatite disk, acid-etched treatment; HP, Hydroxyapatite disk, primer (Transbond XT primer, 3M, Monrovia, CA, USA) treatment; AD, Orthodontic adhesive disk (Transbond XT, 3M).
*Multiple comparisons were performed by *t*-tests using the Bonferroni correction at a significant level of $\alpha = 0.05$.

Table II. Biofilm development by *Streptococcus mutans* UA159 on various surfaces in biofilm medium supplemented with 20 mM sucrose. The amounts of biofilms were expressed as a colony forming unit (CFU) per unit area (cm^2).

	Surface type ($\times 10^8$ CFU/cm^2)				
Saliva treatment	HA ($M \pm$ SD)	HE ($M \pm$ SD)	HP ($M \pm$ SD)	AD ($M \pm$ SD)	Significance*
No treatment	1.11 ± 0.26	1.45 ± 0.26	0.74 ± 0.25	0.15 ± 0.05	HA, HE (no treatment, surface-adsorbed < fluid-phase)
Surface-adsorbed	1.17 ± 0.55	1.27 ± 0.53	2.67 ± 1.11	0.25 ± 0.18	HP (no treatment, fluid-phase < surface-adsorbed)
Fluid-phase	2.59 ± 0.96	2.46 ± 0.80	1.01 ± 0.30	0.16 ± 0.06	AD (no treatment = fluid-phase = surface adsorbed)

HA, Hydroxyapatite disk, no surface treatment; HE, Hydroxyapatite disk, acid-etched treatment; HP, Hydroxyapatite disk, primer (Transbond XT primer, 3M, Monrovia, CA, USA) treatment; AD, Orthodontic adhesive disk (Transbond, XT, 3M).
*Multiple comparisons were performed by *t* -tests using the Bonferroni correction at a significant level of $\alpha = 0.05$.

in F-UWS. S-UWS can inhibit MS adhesion by decreasing the surface free energy of the underlying materials as previously reported [11]. Such surface modification by saliva-coating may reduce the strength of bacterial adhesion to the substratum, resulting in a decrease in the amount of adherent bacteria. F-UWS may inhibit MS adhesion in a different way, since the cells have an opportunity to directly adhere to the surfaces without the interference of saliva-coating before coating completes. Saliva has been shown to mediate the aggregation of MS by interaction with the cell surface adhesin (antigen I/II family) of MS in the fluid phase [5]. Bacterial aggregation induced by the interaction of F-UWS with MS may facilitate bacterial clearance from surfaces during washing procedures, which may uniformly reduce MS adhesion to the underlying surfaces. This is partly consistent with a previous study, which showed that saliva significantly inhibited bacterial adhesion to hydroxyapatite disks [12].

The different role between S-UWS and F-UWS can be partly explained by the fact that S-UWS

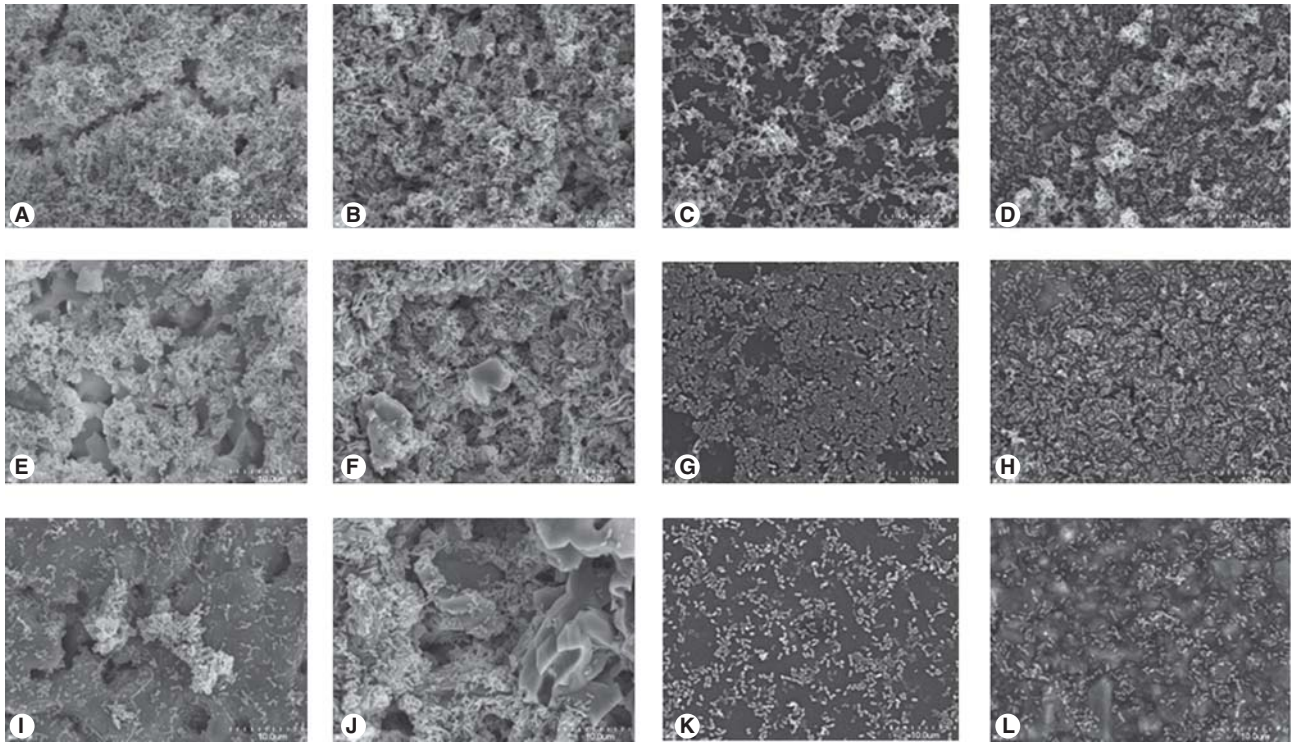

Figure 1. Scanning electron microscopic images of biofilms by *Streptococcus mutans* in the presence of glucose. Hydroxyapatite disk without surface treatment (HA) and no saliva treatment (A); Hydroxyapatite disk with an etched surface (HE) and no saliva treatment (B); Hydroxyapatite disk with a primed surface (HP) and no saliva treatment (C); Orthodontic adhesive (AD) and no saliva treatment (D); HA with adsorbed saliva (E); HE with adsorbed saliva (F); HP with adsorbed saliva (G); AD with adsorbed saliva (H); HA with fluid-phase saliva (I); HE with fluid-phase saliva (J); HP with fluid-phase saliva (K); and AD with fluid-phase saliva (L).

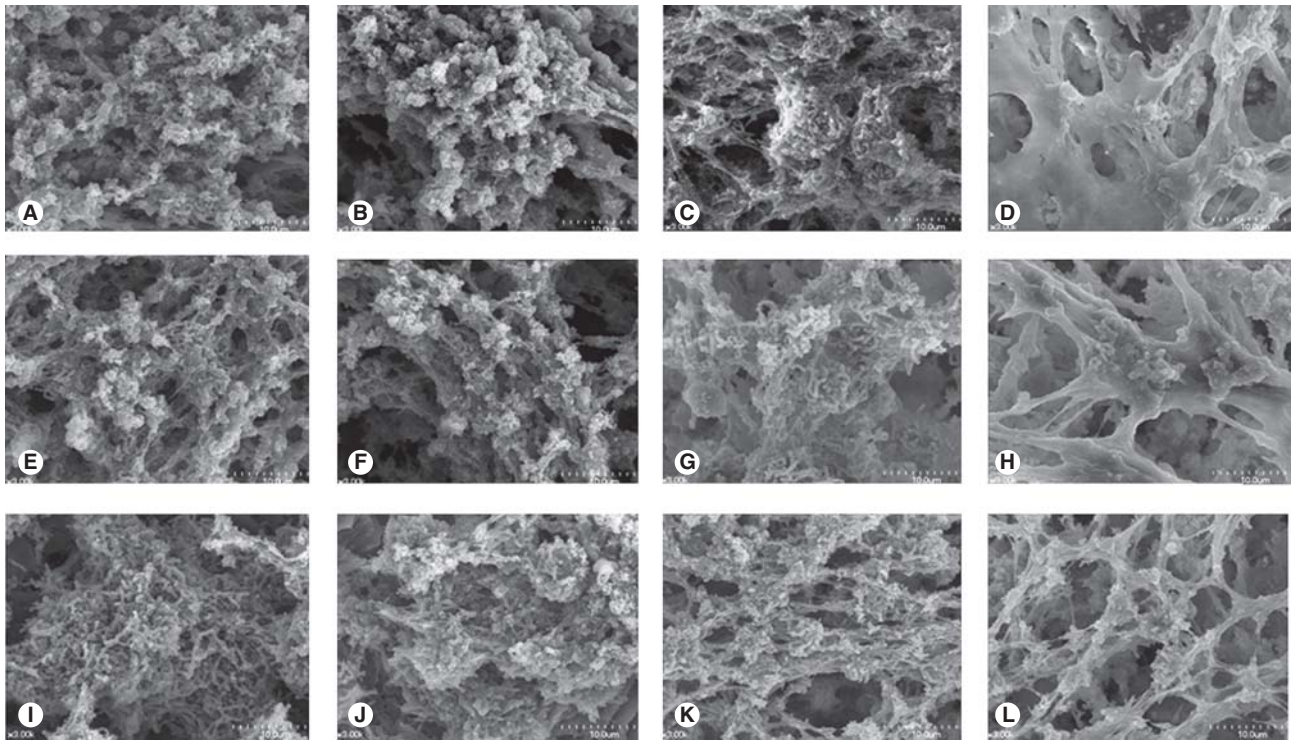

Figure 2. Scanning electron microscopic images of biofilms by *Streptococcus mutans* in the presence of sucrose. Hydroxyapatite disk without surface treatment (HA) and no saliva treatment (A); Hydroxyapatite disk with an etched surface (HE) and no saliva treatment (B); Hydroxyapatite disk with a primed surface (HP) and no saliva treatment (C); Orthodontic adhesive (AD) and no saliva treatment (D); HA with adsorbed saliva (E); HE with adsorbed saliva (F); HP with adsorbed saliva (G); AD with adsorbed saliva (H); HA with fluid-phase saliva (I); HE with fluid-phase saliva (J); HP with fluid-phase saliva (K); and AD with fluid-phase saliva (L).

provides receptors for bacterial binding [5]. Previous studies showed that high-molecular weight mucin and salivary agglutinin mediates the adhesion of *S. mutans* [13,14]. Adsorption of these salivary components to the solid surface can provide sites for adhesion and biofilm formation of *S. mutans*, which may explain that S-UWS resulted in less inhibition of biofilm formation than F-UWS.

In BM-glucose, there were significant differences in biofilm development by *S. mutans* according to the surface type (Table I). Biofilm formation of *S. mutans* was significantly inhibited on HP and AD, compared to HA and HE (HA, HE > HP, AD). The difference in biofilm formation may result from the differences in the physico-chemical properties of HP and AD, which could reduce the strength of attachment of the cells to the substratum. It is also possible that some components of or leached from the primer and adhesive can impair adhesion or biofilm development by *S. mutans*. This can be attributed to the fact that primers or adhesive resins do not exist in a state of chemical inertness in the biological environment of the oral cavity [15,16].

Both UWS treatments did not inhibit biofilm formation of *S. mutans* in BM-sucrose (Table II). This is due to the production of water-insoluble glucans by *S. mutans* in a sucrose-containing medium. One of the most important virulence factors of *S. mutans* is to its production of extracellular polysaccharides (glucans) from sucrose via GTFs [17]. The glucans produced make extracellular layers that promote adhesion and biofilm formation, independent of the presence of saliva. This was confirmed by SEM examinations. Biofilms in BM-sucrose showed thick cell aggregates around an enriched polysaccharide matrix, regardless of the presence of saliva (Figure 2).

In addition, F-UWS or S-UWS uniquely influenced biofilm development in BM-sucrose. Biofilm formation on HA and HE was not promoted by S-UWS, but significantly promoted by F-UWS

Table III. Surface roughness (μm) of various surface used in this study.

	HA ($M \pm$ SD)	HE ($M \pm$ SD)	HP ($M \pm$ SD)	AD ($M \pm$ SD)	Significance*
Surface roughness ($M \pm$ SD)	2.63 ± 0.27	9.20 ± 3.58	3.38 ± 0.89	0.88 ± 0.12	HE > HA, HP > AD

HA, Hydroxyapatite disk, no surface treatment; HE, Hydroxyapatite disk, acid-etched treatment; HP, Hydroxyapatite disk, primer ± Transbond XT primer, 3M, Monrovia, CA) treatment; AD, Orthodontic adhesive disk ± Transbond XT, 3M.
*Multiple comparisons were performed by *t*-tests using the Bonferroni correction at a significant level of $\alpha = 0.05$.

(Table II). This is due to the interactions between F-UWS and GTFs. In the presence of sucrose, saliva is reported to promote the uptake of GTFs and enhance glucan synthetic activities by the aggregation of GTFs [18]. F-UWS in the media can contact GTFs around *S. mutans* cells during biofilm development, which may promote biofilm formation in the presence of F-UWS. In the case of S-UWS, however, a portion of the salivary components can contact GTFs only on the surfaces, which may be the reason for less biofilm formation in the presence of S-UWS than F-UWS.

However, biofilm formation on HP was significantly promoted by S-UWS, but not by F-UWS, compared to the no-UWS treatment group. This may be associated with the interactions of S-UWS and primer components on the HP surfaces. Transbond XT primer consists of two monomer components, trieylene glycol dimethacrylate (TEGDMA) (45–55%) and bisphenol A diglycidyl methacrylate (Bis-GMA) (45–55%) (data obtained from the manufacturer). Previous studies have shown that polymerized TEGDMA and Bis-GMA can be degraded on the surfaces by salivary hydrolysis [19] and the degraded monomers, such as TEGDMA and Bis-GMA, can stimulate GTF activity in sucrose-mediated production of glucans of mutans streptococci [20]. The degradation of the primer layer by S-UWS during or after UWS coating may enhance glucan synthesis and biofilm formation of *S. mutans* on the HP surfaces. F-UWS also increased biofilm formation compared to the control without UWS treatment, but the increased biofilm amount by F-UWS was lower than S-UWS. This may be due to the fact that F-UWS may have less opportunities to react with the primer components on the HP surfaces than S-UWS.

S. mutans showed a significant decrease in biofilm development on AD surfaces even in BM-sucrose, compared to its biofilm formation on other surfaces. In addition, biofilm development on the AD surfaces was not influenced by either UWS treatments. Unknown components from the adhesive may prevent cell growth and/or biofilm formation of *S. mutans*, regardless of a carbohydrate source. Considering that the degraded monomers can stimulate GTF activity in a medium containing sucrose, more residual monomers (due to a lesser degree of polymerization) of primer [21] can partly explain more biofilm formation on the HP surfaces than on the AD surfaces. Further studies will be needed to clarify what components of orthodontic adhesives significantly inhibit biofilm formation of *S. mutans*.

It is interesting that acid-etching did not increase biofilm formation despite causing the roughest surface (Table III). Prior to this study, we expected a close correlation between surface roughness and increased biofilm formation, because a rough surface provides opportunities for bacterial colonization by increasing the adhesion area and preventing dislodgement of bacterial colonies. In addition, we found an increase in the initial adhesion of *S. mutans* to HE surfaces compared to HA surface (data not shown). However, no obvious relationship was observed. This may be due to the fact that biofilm formation includes cell-to-cell interaction as well as cell-to-surface adhesion. The cell-to-cell interaction may be less influenced by surface texture than the cell-to-surface interaction. This may be partly attributed to the effects of etchants under the microgrooves or microridges of the etched surfaces and the existence of other factors affecting bacterial adhesion.

We did the same biofilm experiment using *S. sobrinus* ATCC 33478. However, we observed the same biofilm formation patterns of *S. sobrinus* on the various surfaces used in this study compared to those of *S. mutans*, although the amounts of biofilm formation were lower in *S. sobrinus* than in *S. mutans* (data not shown).

The human mouth, with its diverse niches and environmental changes, is well known for its unrestricted formation of natural microbial biofilms. Although *S. mutans* is a member of the endogenous oral microflora and a major contributor to biofilms in the oral cavity, *S. mutans* cells do not occur in monoculture *in vivo*, but rather in a diverse microbial consortium comprised of several 100 species of bacteria. Further study will be needed to be evaluated in an *in vivo* model to develop a promising approach for prevention of enamel demineralization around orthodontic appliance.

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References

[1] Atack NE, Sandy JR, Addy M. Periodontal and microbiological changes associated with the placement of orthodontic appliances. A review. J Periodontol 1996;67:78–85.

[2] Mitchell L. Decalcification during orthodontic treatment with fixed appliances–an overview. Br J Orthod 1992;19: 199–205.

[3] Artun J, Brobakken BO. Prevalence of carious white spots after orthodontic treatment with multibonded appliances. Eur J Orthod 1986;8:229–34.

[4] Gorelick L, Geiger AM, Gwinnett AJ. Incidence of white spot formation after bonding and banding. Am J Orthod 1982;81: 93–8.

[5] Ahn SJ, Ahn SJ, Wen ZT, Brady LJ, Burne RA. Characteristics of biofilm formation by Streptococcus mutans in the presence of saliva. Infect Immun 2008;76:4259–68.

[6] Mitchell TJ. The pathogenesis of streptococcal infections: from tooth decay to meningitis. Nat Rev Microbiol 2003;1: 219–30.
[7] Koga T, Asakawa H, Okahashi N, Hamada S. Sucrose-dependent cell adherence and cariogenicity of serotype c Streptococcus mutans. J Gen Microbiol 1986;132:2873–83.
[8] Zachrisson BU. Bonding in orthodontics. In: Graber TM VR, editor. Orthodontics: current principles and techniques. St Louis, MO: MosbyYear Book; 1994. p 542–626.
[9] Lim BS, Lee SJ, Lee JW, Ahn SJ. Quantitative analysis of adhesion of cariogenic streptococci to orthodontic raw materials. Am J Orthod Dentofacial Orthop 2008;133:882–8.
[10] Ajdic D, McShan WM, McLaughlin RE, Savic G, Chang J, Carson MB, et al. Genome sequence of Streptococcus mutans UA159, a cariogenic dental pathogen. Proc Natl Acad Sci USA 2002;99:14434–9.
[11] Quirynen M, Bollen CM. The influence of surface roughness and surface-free energy on supra- and subgingival plaque formation in man. A review of the literature. J Clin Periodontol 1995;22:1–14.
[12] Yang IH, Lim BS, Park JR, Hyun JY, Ahn SJ. Effect of orthodontic bonding steps on the initial adhesion of mutans streptococci in the presence of saliva. Angle Orthodontist 2011;81:326–33.
[13] Gibbons RJ, Cohen L, Hay DI. Strains of Streptococcus mutans and Streptococcus sobrinus attach to different pellicle receptors. Infect Immun 1986;52:555–61.
[14] Jenkinson HF, Demuth DR. Structure, function and immunogenicity of streptococcal antigen I/II polypeptides. Mol Microbiol 1997;23:183–90.
[15] Khalichi P, Singh J, Cvitkovitch DG, Santerre JP. The influence of triethylene glycol derived from dental composite resins on the regulation of Streptococcus mutans gene expression. Biomaterials 2009;30:452–9.
[16] Singh J, Khalichi P, Cvitkovitch DG, Santerre JP. Composite resin degradation products from BisGMA monomer modulate the expression of genes associated with biofilm formation and other virulence factors in Streptococcus mutans. J Biomed Mater Res A 2009;88:551–60.
[17] Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev 1986;50:353–80.
[18] Schilling KM, Bowen WH. The activity of glucosyltransferase adsorbed onto saliva-coated hydroxyapatite. J Dent Res 1988; 67:2–8.
[19] Santerre JP, Shajii L, Leung BW. Relation of dental composite formulations to their degradation and the release of hydrolyzed polymeric-resin-derived products. Crit Rev Oral Biol Med 2001;12:136–51.
[20] Kawai K, Tsuchitani Y. Effects of resin composite components on glucosyltransferase of cariogenic bacterium. J Biomed Mater Res 2000;51:123–7.
[21] Hamouda IM A-KA, El Shami FM. Degree of conversion and antimicrobial activity of etch-and-rinse versus self-etching adhesives. J Adhes Dent 2010;12:33–8.