©Operative Dentistry, 2012, **37-5,** 532-539

The Effects of Surface Roughness of Composite Resin on Biofilm Formation of *Streptococcus mutans* in the Presence of Saliva

JW Park • CW Song • JH Jung
SJ Ahn • JL Ferracane

Clinical Relevance

Surface topography (size and depth of depressions) plays a more important role than surface roughness in biofilm formation of *Streptococcus mutans* in the presence of saliva.

SUMMARY

The purpose of this study was to investigate the effects of surface roughness of resin composite on biofilm formation of *Streptococcus mutans* in the presence of saliva. To provide uniform surface roughness on composites, disks were prepared by curing composite against 400-grit silicon carbide paper (SR400), 800-grit silicon carbide paper (SR800), or a glass slide (SRGlass). The surface roughness was examined using confocal laser microscopy. For biofilm formation, *S. mutans* was grown for 24 hours with each disk in a biofilm medium with either glucose or sucrose in the presence of fluid-phase or surface-adsorbed saliva. The adherent bacteria were quantified via enumeration of the total viable counts of bacteria. Biofilms were examined using scanning electron microscopy. This study showed that SR400 had deeper and larger, but fewer

†Jeong-Won Park, DDS, MS, PhD, Yonsei University College of Dentistry, Conservative Dentistry, Gangnam Severance Hospital, Seoul, Republic of Korea

†Chang-Won Song, DDS, MSD, Yonsei University, Department of Dentistry, Seoul, Republic of Korea

Ji-Hye Jung, Korea University, Stem Cell Research, Seoul, Republic of Korea

*Sug-Joon Ahn, DDD, PhD, Seoul National University, Dental Research Institute and Department of Orthodontics, Seoul, Republic of Korea

Jack L Ferracane, EBM, PhD, Oregon Health & Science University, Restorative Dentistry, Portland, OR, USA

†Authors contributed equally to this work.

*Corresponding author: 28-22 Yeongeondong, Jongro-gu, Seoul 110–768, Republic of Korea; e-mail: titoo@snu.ac.kr

DOI: 10.2341/11-371-L

depressions than SR800. Compared to SRGlass and SR800, biofilm formation was significantly increased on SR400. In addition, the differences in the effect of surface roughness on the amount of biofilm formation were not significantly influenced by either the presence of saliva or the carbohydrate source. Considering that similar differences in surface roughness were observed between SR400 and SR800 and between SR800 and SRGlass, this study suggests that surface topography (size and depth of depressions) may play a more important role than surface roughness in biofilm formation of *S. mutans*.

INTRODUCTION

Although the use of resin composites in restorative dentistry is common, there are still several drawbacks to their use, such as susceptibility to plaque accumulation.[1] The resultant increase in plaque retention places patients at higher risk for secondary caries adjacent to the composites.

The major causative microorganism in the pathogenesis of dental caries in humans is *Streptococcus mutans*.[2] Adhesion and/or biofilm formation by *S. mutans* is mediated by sucrose-dependent and sucrose-independent mechanisms.[3] Sucrose-independent binding is mediated by several surface adhesins that can bind to salivary components formed on the teeth in the absence of sucrose.[4] In addition, saliva can mediate the aggregation of *S. mutans* by interaction with the cell surface adhesin (antigen I/II family) of *S. mutans* in the fluid phase.[3] On the contrary, sucrose-dependent adherence is mediated by glucan binding proteins and water-insoluble glucans produced from sucrose by glucosyltransferase enzymes (GTFs).[5] In particular, the formation of glucan matrices can enhance the virulence of the biofilm through increased total biomass and acidogenicity.[6,7]

Surface roughness has been reported to play a prominent role in biofilm formation of oral bacteria.[8,9] The effects of surface roughness on biofilm formation can be explained by the fact that a rough surface can function as a buffer against shear force and can increase the area available for biofilm formation.[9] On the contrary, other studies have reported insignificant relationships between the surface roughnesses of dental materials and the amount of biofilm formation.[10,11] These contradictions mainly may be due to the fact that the surface roughness of the dental materials was not carefully controlled. In most studies, the surfaces were roughened using various polishing techniques with rotating burs or sandpapers,[12–14] resulting in irregular surface roughness, because the time and manual pressure were difficult to control. In addition, the relationship between surface roughness and biofilm formation on composite surfaces can vary because the original differences in surface properties between the dental materials could be masked by the presence of saliva[10,15] or extracellular glucans produced by *S. mutans*.[11]

If the differences in surface roughness, saliva, and extracellular glucans are carefully controlled, the relationships between the surface roughness and biofilm formation of *S. mutans* may be more clearly defined. The purpose of this study was to investigate the effects of surface roughness of composites on biofilm formation of *S. mutans* with different carbohydrate sources in the presence of saliva. The hypothesis of this experiment was that the surface roughness of composite would not affect the biofilm formation of *S. mutans*.

MATERIALS AND METHODS

Materials

The nanofilled composite, Filtek Z350 (A2 shade, 3M ESPE, St Paul, MN, USA) was used in this study. To obtain uniform surface roughness, Teflon molds (9.0 mm in diameter and 1.0 mm in thickness) were placed on silicon carbide (SiC) papers or a glass slide. For the roughest surface group (SR400), disks were prepared on 400-grit SiC paper (Deerfos, Inchon, Korea). Disks were prepared on a glass slide for the smoothest surface group (SRGlass) and on 800-grit SiC paper (Deerfos) for the intermediately rough surface group (SR800). Experimental materials were placed into the hole in the mold until they were flush with the top of the template. A glass slide was placed on top, pushed down to assure flat dorsal surfaces, and then gently removed. The materials were handled according to the manufacturer's instruction and light cured for 40 seconds at 1000 mW/cm^2 using a LED light curing unit (Freelight2, 3M ESPE).

Measurements of Surface Roughness

The surface roughness of each sample was measured using confocal laser scanning microscopy (Axiovert 200M, Carl Zeiss, Thornwood, NY, USA). The multi-argon laser emits light with a wavelength of 633 nm, and it allows the calculation of the arithmetic mean surface roughness from a mean plane within the sampling area (245 $\times$ 245 $\times$ 60 μm). Surface roughness readings were performed on five different areas of each disk.

Saliva Collection

Unstimulated whole saliva (UWS) was collected from healthy volunteers who had no acute dental caries or periodontal lesions. Saliva collection was routinely performed between 9:00 AM and 11:00 AM to minimize the effects of diurnal variability on salivary composition. The saliva sample was centrifuged at 3500*g* for 10 minutes to remove any cellular debris, and the resulting supernatant was used after filter-sterilization through a Stericup & Steritop (Millipore, Billerica, MA, USA).

Biofilm Formation

Overnight cultures of *S. mutans* (serotype c UA 159) were transferred to a prewarmed brain heart infusion (BHI) broth and grown at 37°C in a 5% CO_2 aerobic atmosphere to the late exponential phase ($OD_{600} = 0.5$, approximately 6.5×10^7 colony forming units/mL). The cultures were then diluted 1:100 in a prewarmed biofilm medium (BM) with either 20 mM glucose (BM-glucose) or 20 mM sucrose (BM-sucrose) as a carbohydrate source as previously described.[16] Biofilm assays were performed using three different UWS treatments: fluid-phase UWS (F-UWS), surface-adsorbed UWS (S-UWS), or no UWS treatment (control).

For experiments with F-UWS, 2.0 mL of the diluted cell cultures were inoculated into the well containing a disk, concurrent with 200 μL of UWS. For the experiments with S-UWS, each disk was conditioned with 1.0 mL of UWS in the well at 37°C for 2 hours with gentle shaking, followed by three washes with phosphate buffered saline (PBS). After air drying for 30 minutes, 2.0 mL of the diluted cell cultures (without UWS) were inoculated into the well containing the disk. Two milliliters of cell suspension were inoculated to wells containing a disk without any UWS treatment for control. Biofilms were allowed to form during incubation at 37°C in a 5% CO_2 for 24 hours. 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 via sonication using four 30-second pulses at 25 W with three 30-second intermittent cooling stages in a chilled ice box. The cell suspensions were serially diluted, plated on BHI agar, and incubated at 37°C in a 5% CO_2 atmosphere for two days before the colonies were counted. Colony counts were expressed as colony forming units per unit area (cm^2). All assays were performed in duplicate and independently repeated six times (n=6).

Microscopic Image

Biofilms that were examined using scanning electron microscopy (SEM), were grown as described above and analyzed at 1000× using a S-4700 microscope (Hitachi, Tokyo, Japan).

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. Multiple comparisons were performed with *t* tests using the Bonferroni correction to compare differences between the groups. Surface roughness was analyzed using one-way ANOVA, followed by Tukey honestly significant difference test. All values were considered significant when $\alpha<0.05$.

RESULTS

Comparison of Surface Roughness

There were significant differences in surface roughness between the groups. As expected, the decreasing order of surface roughness was SR400 (1.27 ± 0.06 μm), SR800 (0.80 ± 0.08 μm), and SRGlass (0.29 ± 0.05 μm) (SR400 > SR800 > SRGlass, $p<0.05$). However, the mean variation in surface roughness in each group was very small (less than 0.08 μm), indicating that uniform surface roughness was obtained. The SEM images showed very smooth appearances without any defects on SRGlass (Figure 1G,H,I). In contrast, the other groups showed rough surfaces with various sizes of depressions. SR400 showed deeper and larger depressions (Figure 1A,B,C) than did SR800 (Figure 1D,E,F) which showed more abundant depressions than did SR400.

Biofilm Formation in the Presence of Glucose

When glucose was used as the sole carbohydrate source, both F-UWS and S-UWS significantly suppressed biofilm development by *S. mutans*, compared to that in the control group (Table 1). In addition, S-UWS resulted in greater inhibition of biofilm formation than did F-UWS (no UWS treatment > F-UWS > S-UWS). There were also significant differences in biofilm development by *S. mutans* according to the surface type. Compared to SRGlass and SR800, biofilm formation was significantly enhanced on SR400 in BM-glucose (SRGlass, SR800 < SR400).

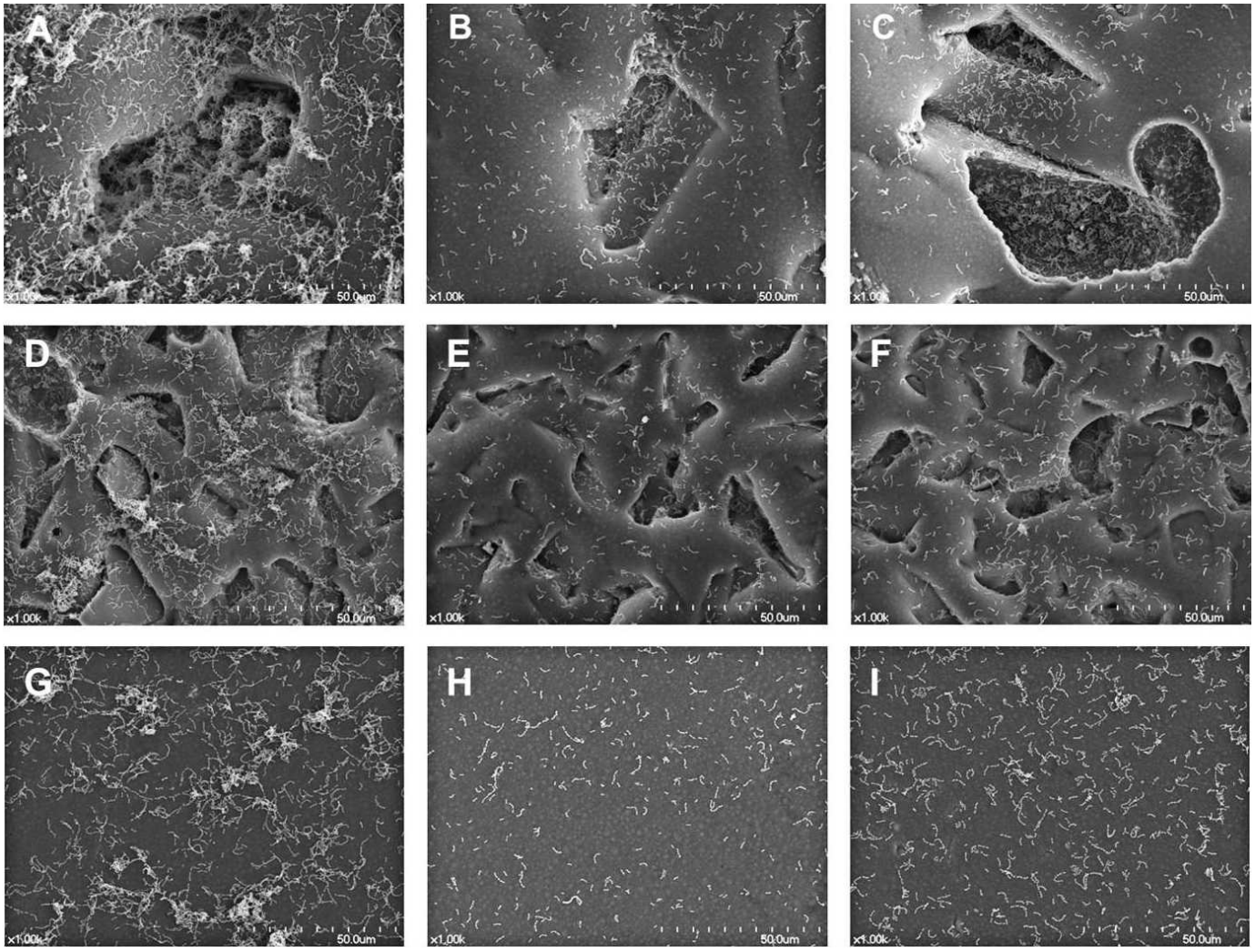

Figure 1. *Scanning electron microscopic images of biofilms of* Streptococcus mutans *formed on various composite surfaces in the presence of glucose. (A): Composite surface prepared against 400-grit silicon carbide (SiC) paper with no saliva treatment. (B): Composite surface prepared against 400-grit SiC paper with surface-adsorbed saliva. (C): Composite surface prepared against 400-grit SiC paper with fluid-phase saliva. (D): Composite surface prepared against 800-grit SiC paper with no saliva treatment. (E): Composite surface prepared against 800-grit SiC paper with surface-adsorbed saliva. (F): Composite surface prepared against 800-grit SiC paper with fluid-phase saliva. (G): Composite surface prepared against a glass slide with no saliva treatment. (H): Composite surface prepared against a glass slide with surface-adsorbed saliva. (I): Composite surface prepared against a glass slide with fluid-phase saliva. SR400 showed deeper and larger depressions (Figure 1A,B,C) than did SR800 (Figure 1D,E,F) which showed more abundant depressions than did SR400. (Data presented here are representative of three independent experiments)*

Biofilm Formation in the Presence of Sucrose

Biofilm patterns in BM-sucrose were different from those in BM-glucose (Table 2). Neither UWS treatments inhibited biofilm development in BM-sucrose. In addition, F-UWS significantly promoted biofilm development on each composite surface. There were also significant differences in biofilm development by *S. mutans* according to the surface type. Similar to biofilm patterns in BM-glucose, *S. mutans* formed more biofilms on SR400 than it did on SR800 or SRGlass in the presence of sucrose.

Comparison of Microscopic Images

Biofilms observed using 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 phases (Figure 1A,D,G) relative to the short scattered chains and microcolonies on the same surfaces in the presence of S-UWS (Figure 1B,E,H) or F-UWS (Figure 1C,F,I). There were also significant differences according to the surface type. In particular, there was an evident

Table 1: *Biofilm Formation by* Streptococcus mutans *on Various Surfaces in the Presence of Glucose for 24 Hours (The Amounts of Bacteria Were Expressed as Colony Forming Units Per Unit Area ($\times 10^7$ CFU/cm^2)*

Saliva Treatment	Surface Type			Significance*
	SR400 (Mean ± SD)[a]	SR800 (Mean ± SD)[b]	SRGlass (Mean ± SD)[c]	
No treatment control	10.65 ± 3.4	8.09 ± 3.01	7.56 ± 1.82	SR400 > SR800, SRGlass control > fluid-phase > surface-adsorbed
Surface-adsorbed	3.37 ± 1.57	2.03 ± 0.63	1.42 ± 0.75	
Fluid-phase	5.54 ± 1.27	4.35 ± 1.50	2.72 ± 0.65	

[a] *The composite surface prepared against 400-grit SiC paper.*
[b] *The composite surface prepared against 800-grit SiC paper.*
[c] *The composite surface prepared against a glass slide.*
* *Multiple comparisons were performed by t-tests using the Bonferroni correction at a significant level of $\alpha=0.05$.*

difference in biofilm formation between SR400 and SRGlass. Abundant cell aggregations were observed in deep and large depressions of rough surfaces (Figure 1A,B,C), while sporadic single cell chains or small cell aggregations were found on smooth surfaces (Figure 1G,H,I). In BM-sucrose, *S. mutans* formed superior biofilms compared to those formed in BM-glucose (Figure 2). Thick cell aggregates were found in abundant polysaccharide matrices, making it difficult to observe differences in microscopic characteristics among the specimens.

DISCUSSION

A number of studies have investigated the amount of biofilm formation on various materials with different surface roughness. In most of those studies, the surfaces of dental materials were subjected to various polishing techniques using rotating burs or sandpapers in order to create differences in surface roughness.[12-14] However, despite using the same polishing tools, heterogeneous surfaces on the specimens themselves and even among specimens in the same group occurred if the time and manual pressure required to polish the surface were not adequately controlled. In contrast to previous studies, we controlled surface roughness by curing the composite material against a glass slide or SiC papers that had been manufactured with homogeneous abrasives. As a result, three different surfaces with uniform surface roughnesses (less than 0.08 μm of the mean variation) were obtained and confirmed using confocal laser scanning microscopy. The three different surfaces were chosen to produce groups that had surface roughness that varied by more than 0.4 μm because more minor variations in surface roughness (less than 0.2 μm) have been shown to have no significant effect on bacterial adhesion.[9,17]

Table 2: *Biofilm Formation by* Streptococcus mutans *on Various Surfaces in the Presence of Sucrose for 24 Hours (The Amounts of Bacteria Were Expressed as Colony Forming Units Per Unit Area ($\times 10^8$ CFU/cm^2)*

Saliva Treatment	Surface Type			Significance*
	SR400 (Mean ± SD)[a]	SR800 (Mean ± SD)[b]	SRGlass (Mean ± SD)[c]	
No treatment control	3.30 ± 0.70	2.59 ± 0.73	2.02 ± 0.76	SR400 > SR800, SRGlass fluid-phase > control, surface-adsorbed
Surface-adsorbed	2.90 ± 1.42	2.24 ± 0.86	2.06 ± 0.78	
Fluid-phase	5.82 ± 1.83	5.01 ± 2.13	3.89 ± 1.09	

[a] *The composite surface prepared against 400-grit SiC paper.*
[b] *The composite surface prepared against 800-grit SiC paper.*
[c] *The composite surface prepared against a glass slide.*
* *Multiple comparisons were performed by t-tests using the Bonferroni correction at a significant level of $\alpha=0.05$.*

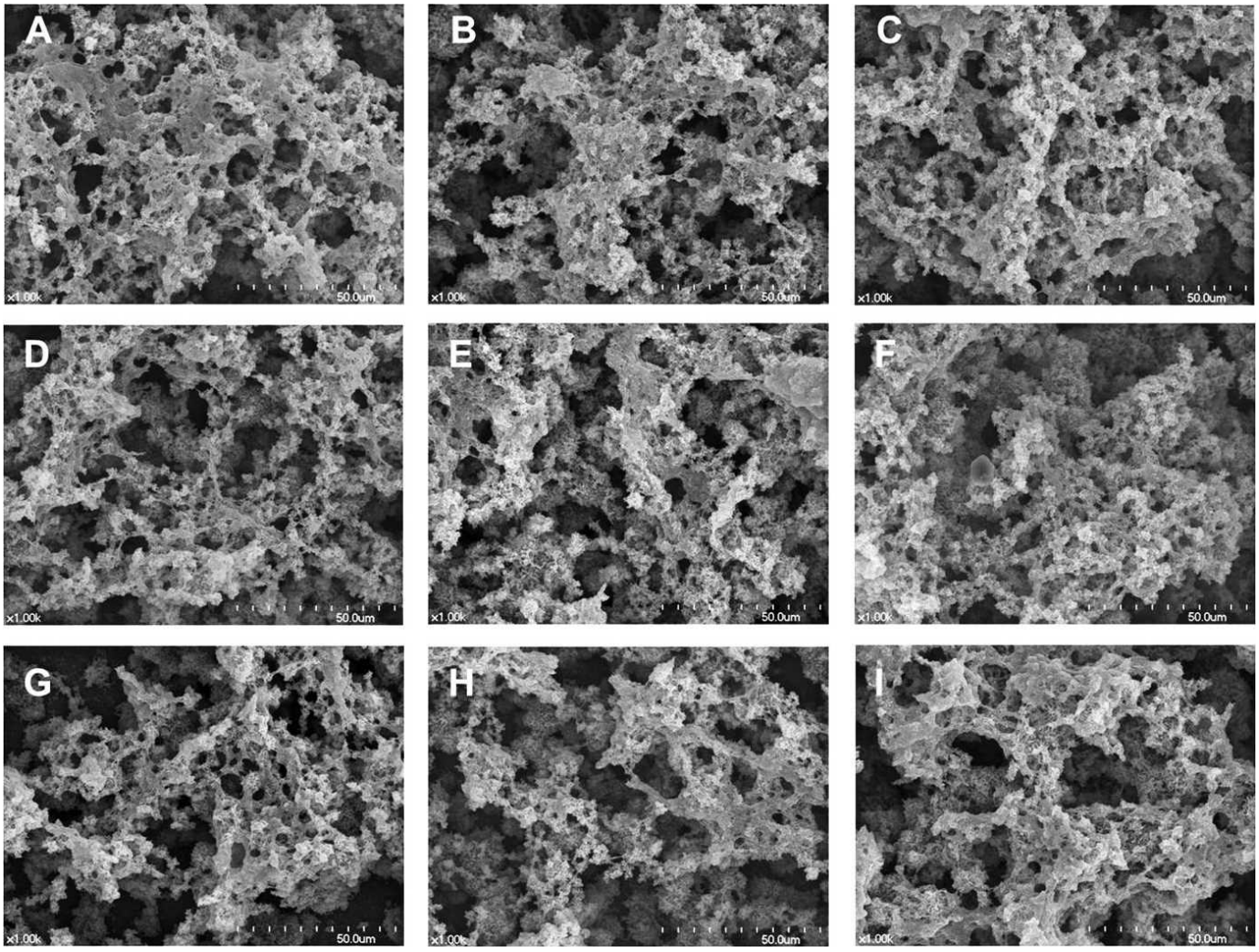

Figure 2. *Scanning electron microscopic images of biofilms of* Streptococcus mutans *formed on various composite surfaces in the presence of sucrose. (A): Composite surface prepared against 400-grit silicon carbide (SiC) paper with no saliva treatment.(B): Composite surface prepared against 400-grit SiC paper with surface-adsorbed saliva. (C): Composite surface prepared against 400-grit SiC paper with fluid-phase saliva. (D): Composite surface prepared against 800-grit SiC paper with no saliva treatment. (E): Composite surface prepared against 800-grit SiC paper with surface-adsorbed saliva. (F): Composite surface prepared against 800-grit SiC paper with fluid-phase saliva. (G): Composite surface prepared against a glass slide with no saliva treatment. (H): Composite surface prepared against a glass slide with surface-adsorbed saliva. (I): Composite surface prepared against a glass slide with fluid-phase saliva. (Data presented here are representative of three independent experiments)*

Biofilm development by *S. mutans* was significantly inhibited by both F-UWS and S-UWS in BM-glucose, with greater inhibition produced by S-UWS (Figure 1; Table 1). This indicates the different roles of UWS in adhesion of *S. mutans* according to its phase. S-UWS may inhibit adhesion of *S. mutans* by decreasing the surface free energy of the underlying material.[9] Surface modification due to 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 adhesion of *S. mutans* in a different way. Saliva has been shown to mediate the aggregation of *S. mutans* through interaction with the cell surface adhesin (antigen I/II family) of *S. mutans* in the fluid phase.[3] Bacterial aggregation induced by the interaction of F-UWS with *S. mutans* may facilitate bacterial clearance from surfaces during washing procedures, which may reduce the adhesion of *S. mutans* to the underlying surfaces. The different roles of S-UWS and F-UWS can be partially explained by the fact that the cells have an opportunity to adhere directly to the surfaces without interference of the saliva-coating in the presence of F-UWS.

In BM-glucose, there were significant differences in biofilm development by *S. mutans* according to the surface type (Table 1), but not by the presence of F-UWS or S-UWS. This demonstrates that the pres-

ence of F-UWS or S-UWS may not significantly modify the effects of surface roughness on the biofilm formation of *S. mutans*.

In BM-glucose, biofilm formation of *S. mutans* was significantly promoted on SR400, compared to that on SR800 or SRGlass. Although not significantly different, biofilm formation tended to increase on SR800 compared to that on SRGlass. The differences in biofilm formation of *S. mutans* between groups can be difficult to explain by the differences in surface roughness, because similar differences in surface roughness were observed between SR400 and SR800 and between SR800 and SRGlass. Instead, the differences in biofilm formation can be explained by the differences in surface topography between the groups. SR400 had deeper and larger depressions on its surface than did SR800 (Figure 1), which may provide more favorable places for bacterial colonization and biofilm formation due to the prevention of the dislodgement of bacterial colonies. Although SR800 showed more abundant depressions than did SR400, the shallower and smaller depressions may provide less favorable places for biofilm formation. This may explain the insignificant differences in the amounts of biofilm formation between SR800 and SRGlass. These results suggest a possibility that surface topography may play a more important role in biofilm formation of *S. mutans* than surface roughness.

In contrast to the biofilm patterns in BM-glucose, neither UWS treatment inhibited the biofilm formation ability of *S. mutans* in BM-sucrose. This may be 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 its production of extracellular polysaccharides (glucans) from sucrose via GTFs.[18] The glucans produce 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 significantly promoted the biofilm formation of *S. mutans* in BM-sucrose (no UWS treatment, S-UWS $<$ F-UWS). This difference may be explained by the interactions between F-UWS and GTFs. In the presence of sucrose, saliva has been shown to promote the uptake of GTFs and to enhance glucan synthetic activities due to the aggregation of GTFs.[19] F-UWS in the media can contact GTFs present around *S. mutans* cells, which may promote biofilm formation. In the case of S-UWS, however, a portion of the salivary components can contact GTFs only on the surface. This may be the reason for reduced biofilm formation in the presence of S-UWS compared to that in the presence of F-UWS. These results may be partially attributed to the effects of enzymatically active GTFs normally present in UWS, which can assist the glucan synthesis of cell-associated GTFs derived from *S. mutans*.[20]

Similar to biofilm patterns in BM-glucose, there were also significant differences in biofilm development by *S. mutans* according to the surface type (Table 2). *S. mutans* formed more biofilms on SR400 than they did on SR800 or SRGlass. In the presence of sucrose, *S. mutans* cells mainly adhere to the surfaces using glucans synthesized by GTFs derived from cell surfaces. Therefore, surface irregularities, specifically larger and deeper depressions, may provide a greater area for glucan accumulation. This drives further development of bacterial colonization and protects bacteria against shear forces during their initial reversible binding, resulting in irreversible and stronger attachment. These results also indicate that surface topography may be an important factor even in the sucrose-dependent binding of *S. mutans* to the underlying surfaces.

It should be noted that in the human oral cavity, complex interactions of a variety of species of oral bacteria with different adhesive and physiologic capacities influence the formation of biofilms on various surfaces. Therefore, *in vitro* experiments do not reflect the complex microbial community found in the oral cavity, although this study allows for determination of the overall effect of surface roughness on microbial physiology and incorporates specific elements associated with the formation of cariogenic biofilms, such as saliva, sucrose, and culture of a proven virulent organism. Further *in vivo* investigation shall elucidate the biologic effects of surface topography on complex bacterial ecology, which will advance our understanding of the biomechanism of natural biofilm formation in the human oral cavity.

CONCLUSIONS

Understanding the relationships among surface roughness, saliva, and biofilm formation of *S. mutans* is important in preventing secondary caries around composite restorations. In this study, influences of surface roughness on biofilm development by *S. mutans* were analyzed in the presence of saliva. Our results showed that surface topography may play a more important role in biofilm formation of *S. mutans* than surface roughness.

Acknowledgements

Supported by a grant of the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare and Family Affairs, Republic of Korea (A091074).

Conflict of Interest Declaration

The Authors of this manuscript certify that they have no proprietary, financial or other personal interest of any nature or kind in any product, service and/or company that is presented in this article.

(Accepted 13 December 2011)

REFERENCES

1. Bernardo M, Luis H, Martin MD, Leroux BG, Rue T, Leitao J, & DeRouen TA (2007) Survival and reasons for failure of amalgam versus composite posterior restorations placed in a randomized clinical trial *Journal of the American Dental Association* **138(6)** 775-783.
2. Loesche WJ (1986) Role of *Streptococcus mutans* in human dental decay *Microbiological Reviews* **50(4)** 353-380.
3. Ahn SJ, Wen ZT, Brady LJ, & Burne RA (2008) Characteristics of biofilm formation by *Streptococcus mutans* in the presence of saliva *Infection and Immunity* **76(9)** 4259-4268.
4. Mitchell TJ (2003) The pathogenesis of streptococcal infections: From tooth decay to meningitis *Nature Reviews. Microbiology* **1(3)** 219-230.
5. Koga T, Asakawa H, Okahashi N, & Hamada S (1986) Sucrose-dependent cell adherence and cariogenicity of serotype c *Streptococcus mutans Journal of General Microbiology* **132(10)** 2873-2883.
6. Shemesh M, Tam A, Feldman M, & Steinberg D (2006) Differential expression profiles of *Streptococcus mutans ftf, gtf* and vicR genes in the presence of dietary carbohydrates at early and late exponential growth phases *Carbohydrate Research* **341(12)** 2090-2097.
7. Duarte S, Klein MI, Aires CP, Cury JA, Bowen WH, & Koo H (2008) Influences of starch and sucrose on *Streptococcus mutans* biofilms *Oral Microbiology and Immunology* **23(3)** 206-212.
8. Hahnel S, Rosentritt M, Burgers R, & Handel G (2008) Surface properties and *in vitro Streptococcus mutans* adhesion to dental resin polymers *Journal of Materials Science. Materials in Medicine* **19(7)** 2619-2627.
9. Quirynen M, & Bollen CM (1995) The influence of surface roughness and surface-free energy on supra- and subgingival plaque formation in man. A review of the literature *Journal of Clinical Periodontology* **22(1)** 1-14.
10. Mei L, Busscher HJ, van der Mei HC, Chen Y, de Vries J, & Ren Y (2009) Oral bacterial adhesion forces to biomaterial surfaces constituting the bracket-adhesive-enamel junction in orthodontic treatment *European Journal of Oral Sciences* **117(4)** 419-426.
11. Shahal Y, Steinberg D, Hirschfeld Z, Bronshteyn M, & Kopolovic K (1998) *In vitro* bacterial adherence onto pellicle-coated aesthetic restorative materials *Journal of Oral Rehabilitation* **25(1)** 52-58.
12. Aykent F, Yondem I, Ozyesil AG, Gunal SK, Avunduk MC, & Ozkan S (2010) Effect of different finishing techniques for restorative materials on surface roughness and bacterial adhesion *Journal of Prosthetic Dentistry* **103(4)** 221-227.
13. Ikeda M, Matin K, Nikaido T, Foxton RM, & Tagami J (2007) Effect of surface characteristics on adherence of *S. mutans* biofilms to indirect resin composites *Dental Materials Journal* **26(6)** 915-923.
14. Yamamoto K, Ohashi S, Taki E, & Hirata K (1996) Adherence of oral streptococci to composite resin of varying surface roughness *Dental Materials Journal* **15(2)** 201-204.
15. Hannig M (1999) Transmission electron microscopy of early plaque formation on dental materials *in vivo European Journal of Oral Sciences* **107(1)** 55-64.
16. Ajdic D, McShan WM, McLaughlin RE, Savic G, Chang J, Carson MB, Primeaux C, Tian R, Kenton S, Jia H, Lin S, Qian Y, Li S, Zhu H, Najar F, Lai H, White J, Roe BA, & Ferretti JJ (2002) Genome sequence of *Streptococcus mutans* UA159, a cariogenic dental pathogen *Proceedings of the National Academy of Sciences of the United States of America* **99(22)** 14434-14439.
17. Bollen CM, Papaioanno W, Van Eldere J, Schepers E, Quirynen M, & van Steenberghe D (1996) The influence of abutment surface roughness on plaque accumulation and peri-implant mucositis *Clinical Oral Implants Research* **7(3)** 201-211.
18. Cross SE, Kreth J, Zhu L, Sullivan R, Shi W, Qi F, & Gimzewski JK (2007) Nanomechanical properties of glucans and associated cell-surface adhesion of *Streptococcus mutans* probed by atomic force microscopy under in situ conditions *Microbiology* **153(Pt 9)** 3124-3132.
19. Schilling KM, & Bowen WH (1988) The activity of glucosyltransferase adsorbed onto saliva-coated hydroxyapatite *Journal of Dental Research* **67(1)** 2-8.
20. Scheie AA, Eggen KH, & Rolla G (1987) Glucosyltransferase activity in human *in vivo* formed enamel pellicle and in whole saliva *Scandinavian Journal of Dental Research* **95(3)** 212-215.