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Microbial changes in biofilms on composite resins with different surface roughness: An in vitro study with a multispecies biofilm model

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ABSTRACT

Statement of problem. The single-species biofilm method cannot represent the interaction and complex functions of microorganisms associated with oral biofilms.

Purpose. The purpose of this in vitro study was to investigate microbial changes in biofilms on composite resins of varying surface roughness by using a multispecies biofilm model with early-colonizing streptococci, middle colonizer, and late-colonizing gram-negative anaerobes.

Material and methods. Composite resin disks were prepared with different roughness: SR180, SR400, SR1500, and SRGlass roughened with 180-, 400-, and 1500-grit silicon carbide paper and glass (control surface without surface roughening). Surface roughness was analyzed by confocal laser scanning and scanning electron microscopy. After multispecies biofilms had been grown on the composite resin surfaces, the adhesion of *Streptococcus mutans*, *Streptococcus sobrinus*, *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and of total bacteria was determined after 1 (T1) and 4 (T2) days. Differences in surface roughness among the 4 groups were tested with 1-way ANOVA. Multifactorial analysis of variance was used to determine the time-related differences in the bacterial composition with respect to surface roughness (α=.05).

Results. The order of SR, from highest to lowest, was SR180 (1.45 ±0.11 μm), SR400 (0.62 ±0.05 μm), SR1500 (0.35 ±0.02 μm), and SRGlass (0.15 ±0.01 μm) (SR180>SR400>SR1500>SRGlass, P<.001). Increased surface roughness was not proportional to bacterial adhesion. Significant differences in the adhesion of total bacteria was only found between SRGlass and SR180 (SR180>SRGlass, P=.029). The adhesion of *S. mutans* and *S. sobrinus* to SR180 and SR400 was higher than that to SRGlass (SR180=SR400>SRGlass; *S. mutans*, P=.003; *S. sobrinus*, P=.002). However, the adhesion of *A. actinomycetemcomitans* and *P. gingivalis* to composite resin was not significantly influenced by surface roughness. Adhesion of total bacteria, *S. mutans*, and *S. sobrinus* increased from T1 to T2 (T1<T2, P<.001), whereas the adhesion of periodontal pathogens decreased from T1 to T2 (T1>T2; *A. actinomycetemcomitans*, P<.001; *P. gingivalis*, P=.013).

Conclusions. Decreased adhesion of cariogenic streptococci and total bacteria was observed at surface roughness values of around 0.15 μm. Periodic finishing of surface roughness should be considered to minimize the adhesion of cariogenic streptococci to composite resin surfaces. (J Prosthet Dent 2019;122:493.e1-e8)

Oral biofilms are associated with oral infectious diseases according to bacterial composition.[1] Cariogenic streptococci (*Streptococcus mutans* and *Streptococcus sobrinus*) are responsible for dental caries,[2] while *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* are a common focus of periodontal research because of their prevalence, influence on pathogenicity, and convenience for experimentation.[3,4]

Composite resin is the most versatile restorative material in contemporary dentistry.[5] Although composite resin is widely used and has numerous advantages, oral biofilms develop more easily on composite resin than on teeth or on other restorative materials.[6] The formation of oral biofilms on the surface of composite resin reduces the longevity of restorations

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Clinical Implications

An increase in surface roughness had a limited effect on biofilm composition. When possible, a periodic finishing to around 0.15 μm of surface roughness should be considered to minimize the adhesion of cariogenic streptococci to composite resin surfaces.

because secondary caries remains the main cause of their failures.[7]

Surface properties, including surface roughness (SR) and surface free energy (SFE), affect oral biofilm formation because they influence the adhesion and retention of oral microorganisms.[8] Although a surface with high SFE thermodynamically promotes bacterial adhesion,[9] SR plays a major role, which exceeds the importance of SFE.[4,10] In particular, a rough surface significantly enhances biofilm formation by increasing the area available for bacterial adhesion and biofilm formation[9] and promotes rapid regrowth by interfering with the cleaning process.[11] In addition, SR affects the composition of multispecies biofilms by increasing the initial adhesion of early colonizers.[4,10] Various finishing and polishing procedures have been introduced to smooth surfaces and to improve the esthetics and longevity of composite resin restorations.[12-19]

Although the relationship between SR and oral biofilm has been investigated,[20-22] the effects of SR on biofilm composition are unclear. This may be because most studies only investigated the adhesion of single bacterial species, mainly *S. mutans*, to composite resin materials.[6,21,22] In addition, few studies have investigated the adhesion of periodontal pathogens to composite resin materials. Furthermore, the clinical SR ranges of oral restorations have not been fully examined.[6,15]

The purpose of this in vitro study was to investigate microbial changes in biofilms on composite resins with varying surface roughness. For this purpose, a 13-species biofilm model, including cariogenic streptococci and periodontal pathogens, and a representative composite resin material with clinically relevant SR ranges and similar SFEs were used. The null hypothesis was that no significant differences would be found in biofilm composition with respect to SR.

MATERIAL AND METHODS

The microhybrid composite resin (Denfil; Vericom Co, Ltd) was used to make specimens for this study. The composite resin was injected into a polytetrafluoroethylene mold (3 mm in thickness and 10 mm in diameter) and packed to evenly fill the mold before a glass slide was put on top. The specimen was light polymerized (B&Lite; B&L Biotech) for 20 seconds from the top and 20 seconds from the bottom. The surface against the glass side was used as the experimental surface in this study. The disks were prepared and assigned to 4 groups: SR180, SR400, SR1500, and SRGlass. Disks in SR180, SR400, and SR1500 were manually roughened with 180-grit, 400-grit, and 1500-grit (CC-180Cw, CC-400Cw, CC-1500Cw; Daesung Abrasive Co, Ltd) wetted silicon carbide (SiC) papers (28-cm long and 23-cm wide). Because 1 side of the surface was only used for biofilm experiments, 1 side of each disk was ground 50 times against a wet SiC paper at a uniform distance (23 cm, the width of SiC papers). Disks in the SRGlass group had a control surface without surface roughening.

The SR was analyzed by confocal laser scanning microscopy (LSM 5 Pascal; Carl Zeiss MicroImaging GmbH) in the sampling area (450×450×50 μm). The measurements were performed at 3 points on each disk. SR was evaluated from the experimental surfaces of all disks for biofilm experiments before biofilm formation. Scanning electron microscopy (SEM) analysis (S-4700 FE-SEM; Hitachi) was used to examine the surface texture of each group at magnifications of ×200 and ×3000.

The SFE can be determined by measuring the contact angle of the drop of a probe liquid.[4] When the water contact angles of the composite resin were measured, there was no significant difference in the contact angle among the 4 SR groups: SR180 (66.2 ±1.3 degrees), SR400 (65.5 ±0.9 degrees), SR1500 (65.5 ±1.4 degrees), and SRGlass (66.3 ±0.5 degrees). These indicate no significant difference in SFE among the 4 surface groups. Therefore, only SR could significantly influence biofilm composition in this study.

A 13-species bacterial consortium was used, chosen as previously described because of their prevalence, metabolic properties, influences on pathogenicity, and convenience for experimentation (Table 1).[4] These bacteria are involved in the process of oral biofilm formation and bacterial intercommunication, which starts with early-colonizers, middle colonizers, and late-colonizing gram-negative anaerobes.[23] After each bacterial strain was grown individually to the late exponential phase as described in Table 2, 1 mL of each culture was combined and gently mixed immediately before the biofilm experiment. A modified McBain medium (MBM) that contained 2.5 g/L of porcine gastric mucin, 2 g/L peptone, 2.5 g/L KCl, 1 g/L yeast extract, 1 g/L trypticase peptone, 0.1 g/L cysteine hydrochloride, 0.001 g/L hemin, 10 mM glucose, and 10 mM urea was used to supply nutrition and to simulate saliva as previously described.[24]

A CDC biofilm reactor (BioSurface Technologies) that could reproduce the supragingival oral environment was used to cultivate the multispecies biofilms.[25] Shear force

Table 1. Thirteen-species bacterial consortium used

Colonizers	Species
Early	*Streptococcus mutans* ATCC 700610
	Streptococcus sobrinus ATCC 27607
	Streptococcus sanguinis CCUG 17826
	Streptococcus salivarius CCUG 50207
	Streptococcus oralis ATCC 9811
	Actinomyces naeslundii KCOM 1472
	Lactobacillus rhamnosus ATCC 7469
Middle	*Veillonella dispar* KCOM 1864
	Fusobacterium nucleatum ATCC 10953
	Neisseria subflava ATCC 49275
Later	*Prevotella nigrescens* ATCC 33563
	Porphyromonas gingivalis KCOM 2797
	Aggregatibacter actinomycetemcomitans ATCC 43718

Table 2. Growth condition of 13-species bacterial consortium used

Bacterial Species Used	Growth Condition
Streptococcus mutans	Brain heart infusion (Becton Dickinson) medium at 37 °C with 5% CO_2 atmosphere
Streptococcus sobrinus	
Streptococcus sanguinis	
Streptococcus salivarius	
Streptococcus oralis	
Actinomyces naeslundii	
Lactobacillus rhamnosus	
Veillonella dispar	
Neisseria subflava	
Fusobacterium nucleatum	After anaerobic condition with tryptic soy agar (Becton Dickinson) medium supplemented with 10 μg/mL of vitamin K, 5 μg/mL hemin, and 5% sheep blood at 37 °C for 7 d, these species were subcultured in BHI medium with 10 μg/mL of vitamin K and 5 μm/mL hemin and grown to midexponential phase anaerobically at 37 °C
Prevotella nigrescens	
Porphyromonas gingivalis	
A. actinomycetemcomitans	BHI (Becton Dickinson) medium at 37 °C in anaerobic condition.

and the constant flow of fresh medium in this biofilm reactor were used to simulate a dynamic oral environment. Each disk was put into a polytetrafluoroethylene cylinder and only exposed to the experimental surfaces to mask the opposite surfaces. After sterilization of the rods with disks, the equipment, and the MBM, the reactor was set on a hot stir plate set at 37 °C with a rotation speed of 60 rpm, as previously described.[4] The consortium of this bacteria mixture was inserted into the biofilm reactor (3.5 mL, 1% of the reactor volume), and a constant flow of MBM was then flushed through the reactor at a rate of 100 mL per hour during the experiment.

To compare time-related biofilm composition with respect to SR, 8 disks (2 sets of 4 different surface groups) were collected from the reactor at 2 time points: days 1 (T1) and 4 (T2). Because, in a previous study,[26] mature biofilms had been found to form in the biofilm reactor after 72 hours, biofilms at T1 and T2 reflect early and mature biofilms. Each disk was transferred into a round tube and washed 2 times with 1.0-mL phosphate-buffered saline (PBS, pH=7.4) to remove unbound bacteria. The biofilm was then detached from the disk by sonication. After removing the disk, the bacterial cell suspension was centrifuged at 13 000 rpm for 10 minutes and washed twice with 1.0 mL of PBS. Bacterial chromosomal DNA was extracted by using a DNA extraction kit (CellEase Bacteria II; Biocosm Inc) according to the manufacturer's instructions.

Known specific PCR primers that amplify the dextranase genes of *S. mutans* and *S. sobrinus* were designed from the *gtfB* and *gtfU* genes.[27] The PCR primers for *P. gingivalis* were designed based on the 16S rRNA gene, and the primers for *A. actinomycetemcomitans* were based on the *rpoB* gene of the RNA polymerase β subunit gene. A conserved sequence in the 16S rRNA gene was selected to quantify the numbers of total bacteria.[27] All primers were commercially synthesized by Bioneer Corp.

The DNA standard curve was obtained from known amounts of purified PCR product. DNA was extracted from *S. mutans* ATCC 700610, *S. sobrinus* ATCC 27607, *P. gingivalis* KCOM 2797, and *A. actinomycetemcomitans* ATCC 43718. PCR products were isolated from agarose gels by using an extraction kit (QIAquick Gel Extraction kit; Qiagen). The amount of bacterial DNA in the specimens was estimated from the standard curve.[28]

Real-time PCR was performed by using the iQ5 system (Bio-Rad Laboratories, Inc). The reaction mixtures contained 2 μL of purified DNA from the disks, a 100-pM primer, and 10 μL of 2× iQ SYBR Green Supermix (Bio-Rad Laboratories, Inc). Distilled water was added to a final volume of 20 μL. Thermocycling conditions are presented in Table 3. PCR data were analyzed by using a software program (iQ5 Optical System Software; Bio-Rad Laboratories, Inc). All the experiments for quantifying bacterial levels were performed in duplicate and independently repeated 5 times.

Differences in SR among the 4 groups were tested with 1-way ANOVA and the Tukey HSD test for multiple comparisons. Multifactorial ANOVA with the Bonferroni correction was used to determine the time-related differences in the bacterial composition with respect to SR (α=.05 for all analyses).

RESULTS

Significant differences in SR were detected among the groups (Table 4). The order of SR, from highest to lowest, was SR180 (1.45 ±0.11 μm), SR400 (0.62 ±0.05 μm), SR1500 (0.35 ±0.02 μm), and SRGlass (0.15 ±0.01 μm) (SR180>SR400>SR1500>SRGlass, P<.001) (Table 4).

SEM images at ×200 magnification showed that the roughened surface had a noticeable increase in narrower grooves with increased SR (Fig. 1A-C) compared with the smooth surface of SRGlass (Fig. 1D). At a magnification of ×3000, surface irregularities gradually increased as SR increased from SRGlass to SR180 (Fig. 1E-H).

Table 3. Primers and thermocycling conditions used

Primer	Sequence (5′-to-3′)	Size of Amplification (Base Pairs)	Initial Denaturation	Denatuation	Annealing	Extension	Cycles
Universal	Forward: TGGAGCATGTGGTTAATTCGA	160	94 °C	95 °C	60 °C	60 °C	40
	Reverse: TGCGGACTTAACCCAACA		30 s	20 s	45 s	10 s	
Streptococcus mutans	Forward: CTACACTTTCGGGTGGCTTG	261	94 °C	95 °C	60 °C	60 °C	40
	Reverse: GAAGCTTTTCACCATTAGAAGCTG		30 s	20 s	45 s	10 s	
Streptococcus sobrinus	Forward: AAAACATTGGGTTACGATTGCG	156	94 °C	95 °C	60 °C	60 °C	40
	Reverse: CGTCATTGGTAGTAGCCTGA		30 s	20 s	45 s	10 s	
Porphylomonas gingivalis	Forward: TGCAACTTGCCTTACAGAGGG	344	95 °C	95 °C	61 °C	72 °C	40
	Reverse: ACTCGTATCGCCCGTTATTC		60 s	5 s	15 s	33 s	
Aggregatibacter actinomycetemcomitans	Forward: GGCGAGCCTGTATTTGATGTGCG	113	95 °C	95 °C	72 °C		40
	Reverse: GTGCCCGGTGGTGCGTCTTTG		10 min	10 s	30 s		

Table 5 shows that both SR and incubation time had significant effects on biofilm compositions, without interaction effects. Significant differences in biofilm adhesion were found among the 4 surface groups with respect to incubation time. The number of total bacteria, *S. mutans*, and *S. sobrinus* increased from T1 to T2 (T1<T2, $P<.001$), while *P. gingivalis* and *A. actinomycetemcomitans* decreased from T1 to T2 (T1>T2; *A. actinomycetemcomitans*, $P<.001$; *P. gingivalis*, $P=.013$).

SR also significantly affected the biofilm composition, but a significant difference in adhesion patterns was found among bacterial species. Significant differences in the adhesion of total bacteria were found only between SRGlass and SR180 (SR180>SRGlass, $P=.029$). The adhesion of *S. mutans* and *S. sobrinus* to SR180 and SR400 was higher than to SRGlass (SR180=SR400>SRGlass; *S. mutans*, $P=.003$; *S. sobrinus*, $P=.002$) (Table 5). However, there was no significant difference in the adhesion of *A. actinomycetemcomitans* and *P. gingivalis* to composite resin with respect to SR.

DISCUSSION

SR significantly affected the biofilm composition; therefore, the null hypothesis was rejected. Although surface roughening significantly influenced bacterial adhesion to composite resin surfaces, an increase in SR was not directly proportional to bacterial adhesion. In this study, the adhesion of *S. mutans* and *S. sobrinus* to SR180 and SR400 was higher than to SRGlass (SR180=SR400>SRGlass; *S. mutans*, $P=.003$; *S. sobrinus*, $P=.002$), while no significant difference in adhesion of *S. mutans* and *S. sobrinus* was found between SR180 or SR400 and SR1500 and between SR1500 and SRGlass (Table 5). These results indicate that an SR value between 0.15 μm (SRGlass) and 0.35 μm (SR1500) is a threshold value for adhesion of *S. mutans* and *S. sobrinus* to composite resin surfaces. This threshold value can be explained by the dimensions of oral bacteria. Early colonizers such as *S. mutans* and *S. sobrinus* may firmly adhere to crack, groove, or abrasion defects during the initial step for biofilm formation because a rougher surface plays a protective role against shear forces and increases the surface areas to which bacteria would adhere.[9] The size of oral bacteria ranges from 0.3 μm to 1.5 μm,[29] specifically, the size of cariogenic streptococci is about 0.3 μm.[30] SEM images of SRGlass showed smooth surfaces without evident irregularities and grooves (Fig. 1D, 1H). However, a noticeable increase was seen in grooves and irregularities with an increase in SR (Fig. 1).

Table 4. Surface roughness differences based on surface treatment conditions

Surface Roughness				
SR180[a]	SR400[b]	SR1500[c]	SRGlass[d]	Significance[e]
1.45 ±0.11	0.62 ±0.05	0.35 ±0.02	0.15 ±0.01	(SR180>SR400>SR1500>SRGlass)*

[a]Composite resin surface roughened by using 180-grit silicon carbide paper. [b]Composite resin surface roughened by using 400-grit silicon carbide paper. [c]Composite resin surface roughened by using 1500-grit silicon carbide paper. [d]Composite resin surface prepared with glass slide. [e]One-way ANOVA analyzed intergroup differences (α=.05); *$P<.001$.

Once the depth and width of grooves and irregularities are larger than the bacterial dimensions, surface grooves and irregularities can enhance bacterial adhesion, specifically adhesion of early colonizers to underlying surfaces. However, an SR that is smaller than the bacterial dimension can have less influence on bacterial adhesion to underlying surfaces. Previous research demonstrated that 0.2-μm SR was a threshold value for bacterial adhesion to underlying surfaces.[13] Bacterial adhesion to dental biomaterials significantly increased from 0.2 μm of SR; however, smoothing below 0.2 μm did not result in further improvement of the microbiological and clinical parameters.[31] Although there were differences in materials used (titanium implant abutment versus composite resin) and experimental design (in vivo versus in vitro multispecies biofilms) between the previous and present studies, the threshold SR value in the previous study is partly consistent with that in the present study.

Table 5 shows an increasing tendency of adhesion of *S. mutans* and *S. sobrinus* with increased SR. Adhesion of *S. mutans* and *S. sobrinus* was significantly increased in

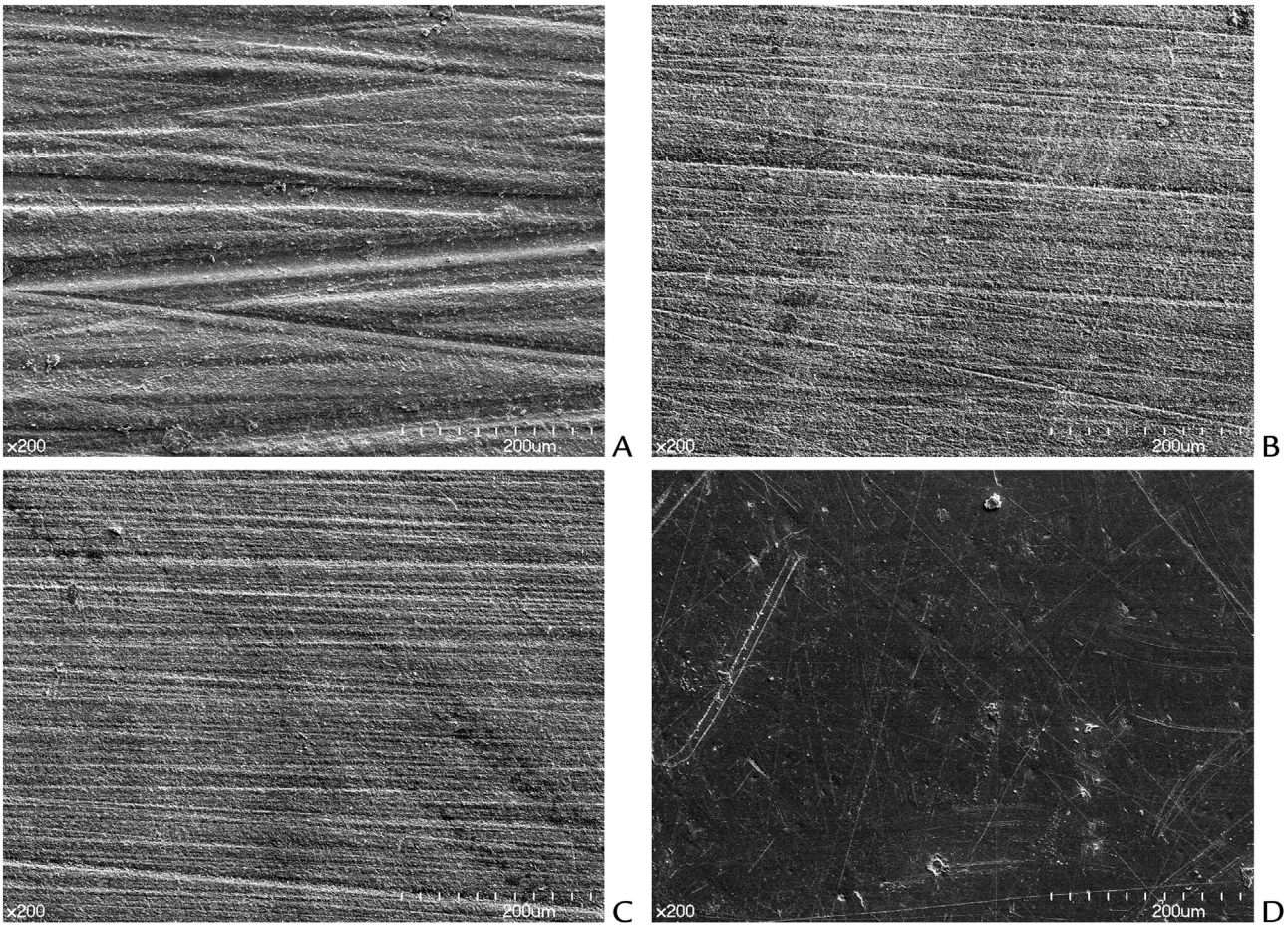

Figure 1. Scanning electron microscopic images of composite resin surfaces. A, E, Prepared against 180-grit silicon carbide paper. B, F, Prepared against 400-grit silicon carbide paper. C, G, Prepared against 1500-grit silicon carbide paper. D, H, Prepared with glass slide. Original magnification A-D, ×300; E-H, ×3000.

composite resin surfaces with an average of 0.62 μm of SR (SR400), but no significant differences were found in bacterial adhesion between SR400 and SR180 (average 1.45 μm). This indicates that an SR over a certain level (around 0.62 μm in the present study) might not significantly influence biofilm composition on composite resin surfaces. In the present study, MBM containing porcine gastric mucin that coats the surface, similar to salivary pellicles, was used.[24] The formation of mucin coating on the roughened surface would mask the physiochemical surface properties of dental materials and alter SR effects.[32] Although a significant difference in SR was noted among the groups, SEM images showed that SR180, SR400, and SR1500 had similar surface texture (Fig. 1). The similar texture could also reduce the differences in biofilm composition with respect to SR change.

Surface roughening did not significantly influence the adhesion of *P. gingivalis* or *A. actinomycetemcomitans* to composite resin surfaces (Table 5). This suggests that SR may have a more significant influence on the adhesion of early colonizers (*S. mutans* and *S. sobrinus*) than on that of the late colonizers (*A. actinomycetemcomitans* and *P. gingivalis*),[9,11] possibly because the late colonizers do not initially colonize on tooth surfaces but adhere to early colonizers that have already adhered to tooth surfaces.[11]

The adhesion pattern of the total bacterial colony was somewhat similar to that of cariogenic streptococci. The adhesion of cariogenic *S. mutans* and *S. sobrinus* was significantly different between SRGlass and SR400 or SR180 (SR180=SR400>SRGlass; *S. mutans*, P=.003; *S. sobrinus*, P=.002), while significant differences in the adhesion of total bacteria were found between SRGlass and SR180 (SR180>SRGlass, P=.029) (Table 5). In general, the formation of multispecies biofilms is part of a complex process and has interspecies interactions. Biofilm formation is not only governed by the initial adherence of early colonizers to the surface of dental biomaterials but also by bacterial interaction.[33] During growth, middle and/or late colonizers adhere to the already present biofilm and not the material surface. Therefore, adhesion of the total bacterial colony may be

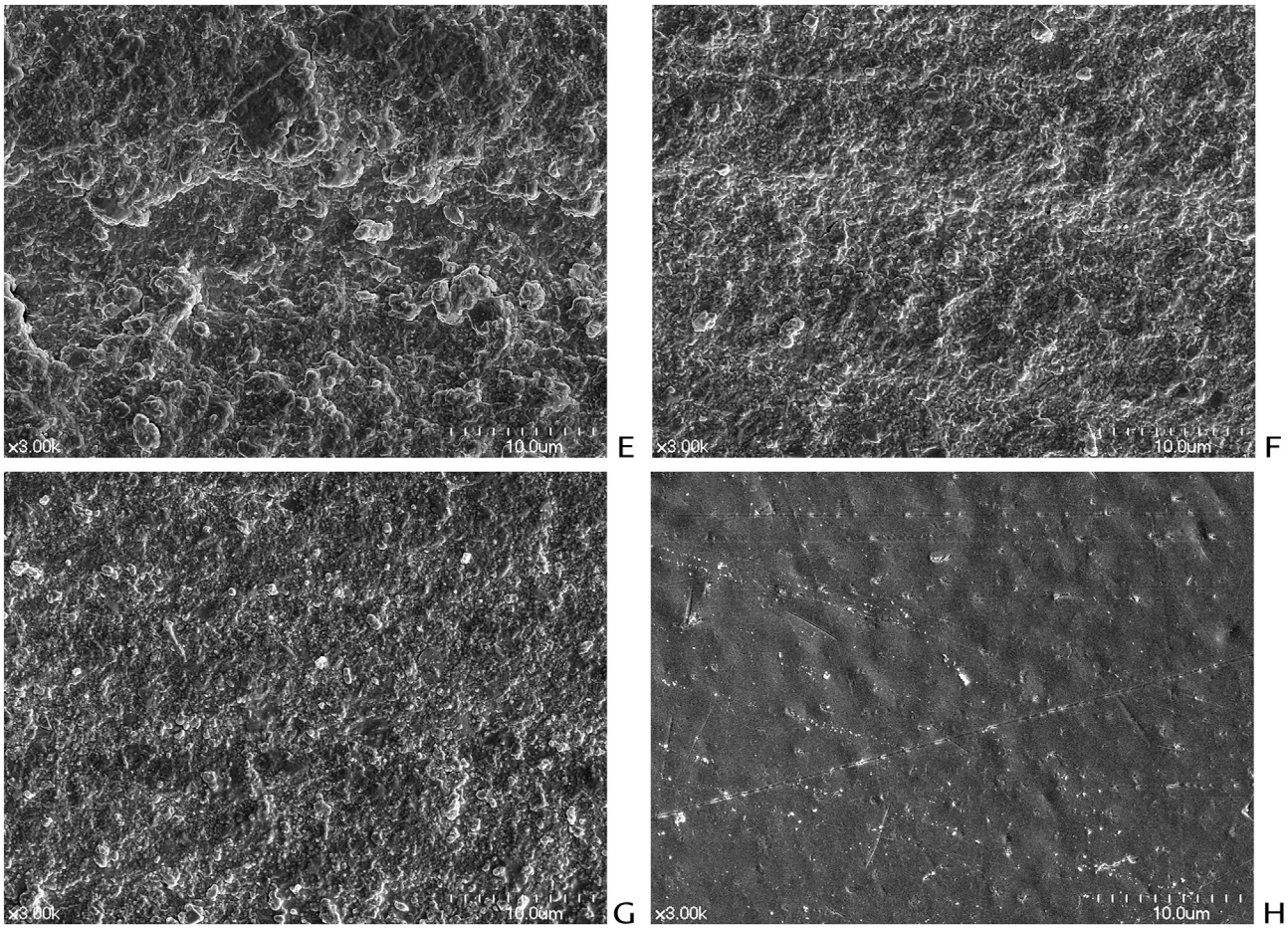

Figure 1. (*continued*).

significantly influenced by early colonizers (streptococci) rather than later colonizers (*A. actinomycetemcomitans* and *P. gingivalis*).

Incubation time also significantly influenced the composition of biofilms (Table 5). The amount of *S. mutans* and *S. sobrinus* in biofilms significantly increased (T1<T2), while that of *P. gingivalis* and *A. actinomycetemcomitans* significantly decreased with extended incubation time (T1>T2). As a biofilm matures, the microenvironment changes from aerobic to anaerobic, and anaerobic bacteria become more prevalent.[34] In the present experiment, however, continuous shear force and a constant flow of fresh medium were provided to simulate the supragingival oral environment with aerobic conditions. Therefore, this experimental design could cause a continuous oxygen supply in the reactor, which can decrease anaerobic bacteria (*P. gingivalis* and *A. actinomycetemcomitans*) and increase facultative bacteria (*S. mutans* and *S. sobrinus*) with extended incubation time. This result is consistent with a previous study.[4]

Finishing and polishing are fundamental steps to improve the esthetics and longevity of composite resin restorations because poorly polished composite resin restorations are susceptible to biofilm formation, gingival irritation, and secondary caries.[12,14,17] In the present study, SRs of SR180, SR400, SR1500, and SRGlass groups (0.15 to 1.45 μm) represented those achieved by clinical finishing and polishing techniques (Table 4): SR180 (around 1.45 μm) represents finishing with diamond rotary instruments at high speed[13,19]; SR400 (around 0.70 μm) represents finishing with white stones[13,18]; SR1500 (around 0.30 μm) represents finishing with 30 fluted tungsten carbide burs[13,16]; and SRGlass (around 0.15 μm) represents finishing with aluminum oxide–graded abrasive flexible disks.[13,15]

Daily tooth brushing and the aging process increase the SR of composite resin restorations by about 0.34 μm,[35,36] which may influence the durability of restorative materials by inducing increased adhesion of cariogenic streptococci. Smoother surfaces can be achieved by reducing filler size,[15] and periodic polishing with, for example, the EP polishing system may reduce the SR of composite resins to below 0.15 μm.[16] The present study suggests that a combination of composite resins with smaller filler sizes and periodic repolishing are recommended to minimize cariogenic biofilm

Table 5. Biofilm composition with respect to surface roughness (μm)

Bacteria	Day 1 (T1)	Day 4 (T2)	Significance[e] Time	Surface Roughness
Total bacteria (Log_{10}/unit area)				
SR180[a]	7.3 ±0.2	7.8 ±0.4	T1<T2 (P<.001)	SR180>SRGlass (P=.029)
SR400[b]	7.2 ±0.2	7.8 ±0.3		
SR1500[c]	7.1 ±0.2	7.7 ±0.4		
SRGlass[d]	7.1 ±0.2	7.6 ±0.4		
Streptococcus mutans (Log_{10}/unit area)				
SR180[a]	3.9 ±0.4	4.2 ±0.6	T1<T2 (P<.001)	SR180=SR400>SRGlass (P=.003)
SR400[b]	3.8 ±0.5	4.1 ±0.5		
SR1500[c]	3.8 ±0.4	4.1 ±0.3		
SRGlass[d]	3.6 ±0.4	3.8 ±0.3		
Streptococcus sobrinus (Log_{10}/unit area)				
SR180[a]	4.1 ±0.2	5.0 ±0.6	T1<T2 (P<.001)	SR180=SR400>SRGlass (P=.002)
SR400[b]	4.1 ±0.4	4.8 ±0.6		
SR1500[c]	3.9 ±0.4	4.8 ±0.6		
SRGlass[d]	3.8 ±0.2	4.4 ±0.6		
Aggregatibacter actinomycetemcomitans (Log_{10}/unit area)				
SR180[a]	4.3 ±0.5	3.9 ±0.4	T1>T2 (P<.001)	SR180=SR400=SR1500=SRGlass (P=.155)
SR400[b]	4.2 ±0.3	3.9 ±0.3		
SR1500[c]	4.1 ±0.2	3.9 ±0.3		
SRGlass[d]	4.0 ±0.2	3.9 ±0.5		
Porphyromonas gingivalis (Log_{10}/unit area)				
SR180[a]	2.2 ±1.0	2.0 ±0.7	T1>T2 (P<.013)	SR180=SR400=SR1500=SRGlass (P=.745)
SR400[b]	2.0 ±1.2	1.7 ±0.9		
SR1500[c]	2.1 ±0.9	1.7 ±0.9		
SRGlass[d]	2.2 ±0.6	1.7 ±0.7		

[a]Composite resin surface roughened by using 180-grit silicon carbide paper. [b]Composite resin surface roughened by using 400-grit silicon carbide paper. [c]Composite resin surface roughened by using 1500-grit silicon carbide paper. [d]Composite resin surface prepared with glass slide. [e]Multifactorial analysis of variance used to determine time-related differences in bacterial composition with respect to surface roughness (α=.05).

development around composite resin surfaces, which may increase restoration longevity and oral health.

In the present study, the unit of bacterial adhesion was converted from exponential form to logarithmic form to simplify the description of the data, which made the differences between smooth and rough surfaces relatively small. However, the actual differences in bacterial adhesion between the surface groups were not too small to be ignored. For example, although the mean difference in adhesion amounts of *S. mutans* between SRGlass and SR400 at day 1 was about 0.2 (Table 4), it indicates that over 2000 ($10^{3.8}$-$10^{3.6}$) bacteria adhered more to SR400 than to SRGlass. Considering that the doubling time of *S. mutans* was less than 1 hour in the presence of a carbohydrate source, that amount may be sharply increased over a short time.

There were some limitations to this study. The specimens prepared in the laboratory were different from those prepared in the clinical environment. In addition, over 500 species are present in the oral cavity, which is also different from our experimental condition. Further study will be required to investigate the effects of SR on biofilm formation in in vivo clinical situations.

CONCLUSIONS

Based on the findings of this in vitro study, the following conclusions were drawn:

1. An increase in surface roughness was not directly proportional to bacterial adhesion.
2. Periodontal pathogens, such as *A. actinomycetemcomitans* and *P. gingivalis*, were not significantly influenced by surface roughness changes.
3. Adhesion of total bacteria and cariogenic streptococci increased, but adhesion of periodontopathogens decreased with extended incubation time.
4. A periodic smoothing to around 0.15 μm is an effective strategy to minimize cariogenic biofilm formation because decreased adhesion of cariogenic streptococci was observed at surface roughness values of around 0.15 μm.

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