Dental Materials Journal 26(5): 613−622, 2007

Surface Properties of Resin Composite Materials Relative to Biofilm Formation

Masahiro ONO[1], Toru NIKAIDO[1], Masaomi IKEDA[1], Susumu IMAI[3], Nobuhiro HANADA[3], Junji TAGAMI[1,2] and Khairul MATIN[1,2]

[1]Cariology and Operative Dentistry, Tokyo Medical and Dental University, Tokyo, Japan
[2]Center of Excellence (COE) Program for Frontier Research on Molecular Destruction and Reconstruction of Tooth and Bone at TMDU, Tokyo, Japan
[3]Department of Oral Health, National Institute of Public Health, Saitama, Japan
Corresponding author, Masahiro ONO; E-mail: ono.ope@tmd.ac.jp

Received September 25, 2006/Accepted March 20, 2007

The surface properties of three different resin composite materials which influence *Streptococcus mutans* biofilm formation were evaluated using an artificial mouth system (AMS). Specimens were prepared from Clearfil AP-X, Grandio, and Reactmer Paste, and each material was divided into two groups: (1) surface was ground with 800-grit silicon paper (SiC＃800); or (2) surface was polished with up to 1-μm diamond paste (DP1 μm). Biofilms were grown on the surface of each specimen for 20 hours, and then subjected to vortex agitation followed by measurement of retained biofilms. Surfaces with retained biofilms were also inspected by SEM. Significant differences were detected in surface roughness (Ra) between the two polishing conditions for all materials. The quantity of retained biofilm was significantly less ($p<0.05$) on Clearfil AP-X DP 1 μm than on Clearfil AP-X SiC＃800. With Reactmer Paste, their surfaces registered the lowest amount of retained biofilm — but there were no significant differences between the two polishing conditions. In conclusion, polishing did not render all resin composites equally resistant to biofilm formation.

Keywords: Resin composite, *S. mutans*, Biofilm adherence

INTRODUCTION

Resin composites have been widely used for anterior and posterior restorations in clinical practice. The latter are often referred to as direct restorations because the materials utilized are called direct resin composites. These types of resin composites play a major role in operative, esthetic, and prosthodontic treatments — especially in minimally invasive restorations. However, one pressing reason for the replacement of direct resin composite restorations is the development of secondary caries which influences the longevity of the restorations[1-4]. One possible causative factor for the development of secondary caries is bacterial adhesion and biofilm formation on the resin composite surface.

Several species of bacteria have been reported to be isolated from plaque associated with caries lesions. *Streptococcus mutans* (*S. mutans*) is one bacterial species most frequently implicated in dental caries[5]. Previously, it has been reported that adherence of oral streptococci was influenced by the composition of resin composites[6]. In addition, a clear correlation has also been noted between surface roughness of resin composites and biofilm adherence[7,8].

There are various types of resin composites with different chemical compositions[9], which may influence the adherence of oral streptococci into the supporting structure of biofilms. The ability of dental materials to inhibit recurrent caries formation is an important clinical property. Their antibacterial activity[10,11] is generally attributed to fluoride release[12-14] and low pH during setting. During the last two decades, numerous resin composites containing fluoridated glass fillers have been developed with fluoride-releasing resin composite being one of those types.

In the process of plaque formation on solid substrate surfaces including teeth and restorative materials, initial adhesion of the "early colonizers" to the surface is a very important step[15,16]. Microscopic examination of early plaque formation on teeth showed adhesion of the initial colonizing bacteria along cracks and pits in enamel, suggesting the influence of surface structure on bacterial adhesion. It is obvious that resin composites have different surface characteristics relative to tooth structures. Moreover, some monomer components of resin might actually stimulate the growth of cariogenic bacterial species[17].

Recently, an "artificial mouth" system (AMS) was developed to study oral biofilm formation on dental materials *in vitro* by simulating the human oral environment. By using the AMS a study model was established to provide a better understanding of how the surface properties of a dental material could influence biofilm adherence and growth.

The influence of fillers, resins, and curing conditions on the properties of dental resin composites has been extensively studied. Previous studies have investigated the effects of filler level on composite properties. Besides, there are also reports on bacterial adherence to composite resins with varying surface roughness[18]. However, little information is available on the surface properties of

light-cured resin composites in relation to biofilm adhesion and growth using a biofilm reactor (such as AMS) which simulates the oral microbial conditions. Against this backdrop of information scarcity, the purpose of this study was to evaluate the surface properties of three different resin composite materials in relation to *S. mutans* biofilm adherence and growth *in vitro* using an AMS.

MATERIALS AND METHODS

Materials used in this study

Direct resin composites used in this study are listed in Table 1. Clearfil AP-X (Kuraray Medical, Tokyo, Japan) and Grandio (Voco, Cuxhaven, Germany) were fluoride-free and Bis-GMA-based resin composites. Reactmer Paste (Shofu, Kyoto, Japan) was a fluoride-releasing UDMA-based resin composite.

Surface roughness analysis

Each resin composite was dispensed from a syringe into a metal mold, and then light-cured for 30 seconds from the top and bottom sides using a visible light curing unit (Optilux 500, Sybron Kerr Corp., USA) to prepare a square-shaped specimen (approximately $4.5 \times 4.5 \times 1.5$ mm). Specimens were then divided into two groups: (1) experimental surface was ground with 800-grit SiC paper (SiC #800); and (2) experimental surface was polished with diamond paste of up to 1 μm particle size (DP1 μm) — and all done under running water. Surface roughness (Ra value) of the specimens of each group was measured using a confocal laser scanning microscope (VK8510, Keyence, Tokyo, Japan).

Preparation of bacterial suspensions

S. mutans MT8148 was used in this study. A suspension of *S. mutans* in phosphate buffered saline (PBS) at $OD_{500} = 2$ (approximately 2×10^7 colony-forming units/ml) was prepared from a 16-hour fresh culture in Brain Heart Infusion (BHI; Becton Dickinson, Sparks, MD, USA) broth after washing three times with PBS and stored at 4℃ with gentle stirring. For growth, a solution of Heart Infusion (HI; Becton Dickinson, Sparks, MD, USA) broth with sucrose (1% final concentration) was used.

Growing biofilm inside AMS

Artificial biofilms were grown on resin composite slab surfaces inside two identical water jacket-encircled chambers of the AMS, as illustrated in Fig. 1. Four slabs from each group were placed on a Teflon holder around a flat bulb pH electrode of the AMS by using red utility wax (GC, Tokyo, Japan). In such a manner, only the experimental surface remained open for biofilm attachment. The open surface of the slab was kept horizontally level to the bulb surface. As for the Teflon holder bearing the slabs, it was set at the bottom opening of the chamber by a silicon plug. Pooled sterile saliva was then poured onto the slab and the electrode, followed by incubation for 30 minutes to obtain a coat of salivary pellicle on the slab surface.

The chamber encircled by the water jacket was sealed with another silicon plug fitted with five stainless steel tubes (21-gauge) so that the chamber itself served as an incubator with a 37℃ inner temperature. The other ends of the five stainless steel tubes were connected to silicon tubes passing through peristaltic pumps regulated by a computer-operated controller (EYALA EPC-2000, Tokyo Rika, Tokyo, Japan). One of them was used to collect the *S. mutans* suspension, two to collect HI, and the other two to collect PBS from the prepared stock as described above. All of these liquids were pumped into the chambers at 6 ml per hour per tube so that they can persistently drop onto the center of the specimen holder. All of these liquids formed water domes which were mixed by the force of gravity exerted from the falling liquid drops on the holder, and then diffusely distributed over all the specimens. When the liquid domes reached their maximum height, the mixture of excess liquids would fall off from the edges of the holders. Both chambers were simultaneously operated, and the pH on the flat bulb electrode was recorded continuously.

Measurement of biofilms

After biofilm formation for 20 hours on the slabs in

Table 1 Materials used in this study

Material (Lot. number)	Manufacturer	Composition
Clearfil AP-X (Lot. 01004A)	Kuraray Medical, Tokyo Japan	barium glass • silanated colloidal silica (filler content 85.0 wt.%) Bis-GMA, TEGDMA
Grandio (Lot. 420394)	Voco, Germany	nano filler (filler content 56.0 wt.%), barium Aluminum silicate (filler content 21.0 wt.%), Bis-GMA, UDMA
Reactmer Paste (Lot. 030405)	Shofu, Kyoto Japan	F-PRG filler • Ultra-fine filler (filler content 78.0 wt.%), catalyst, UDMA, TEGDMA, 2-HEMA,

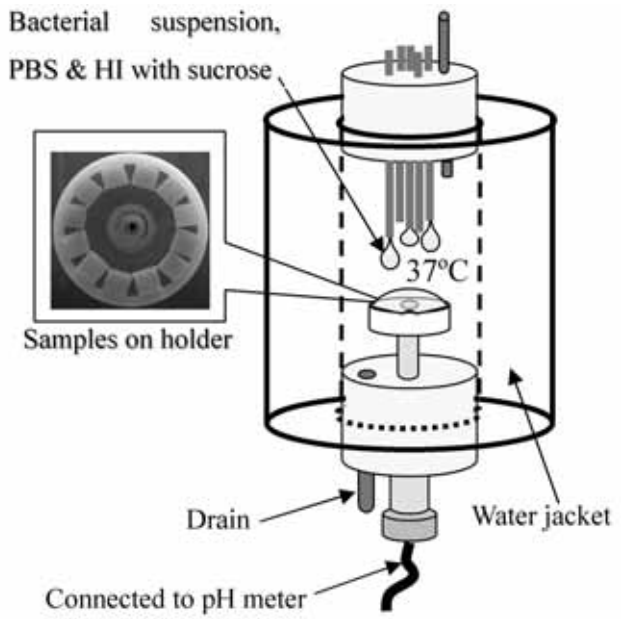

Fig. 1 Diagram represents one of the chambers of AMS, where artificial biofilms were formed on the slab surfaces. Digital photographs were the resin composite slabs before and 5 hours after biofilm formation.

the AMS chamber, each composite slab with artificial biofilm was removed from the Teflon holder and subjected to vortex agitation (Vortex, Scientific Industries, USA) for 15 seconds in cool PBS. Following vortex agitation, retained biofilms were measured after separating the bacterial cells and water-insoluble glucan (WIG), as depicted in Fig. 2. Each slab was transferred carefully from PBS to 1 ml of 0.5 mol/l sodium hydroxide solution, incubated for 15 minutes, vortexed and centrifuged at 5,000 rpm for 10 minutes to separate the WIG and the bacterial cells embedded in the biofilm.

Each bacterial pellet was resuspended in 1 ml of PBS, and 100 μl of each bacterial cell suspension was transferred into separate wells of 96-well flat-bottom microplate to quantify bacteria by turbidimetric analysis (OD_{500nm}) with a Biotrak II plate reader (Biochrom, Cambridge, UK). The amount of dissolved WIG was measured by the phenol-H_2SO_4 method[19] and the absorbance at 492 nm determined with Biotrak II plate reader. The 500-μl WIG solution from each sample was disintegrated by phenol-H_2SO_4, and 200 μl of each resulting solution was analyzed to calculate the amount of WIG (μg/ml). To obtain a standard curve, 0, 25, 50, 75, 100, 150, and 200 μg/ml of glucose, respectively, were quantitated at the same time using the same method. The experiments were repeated three times for reproducibility.

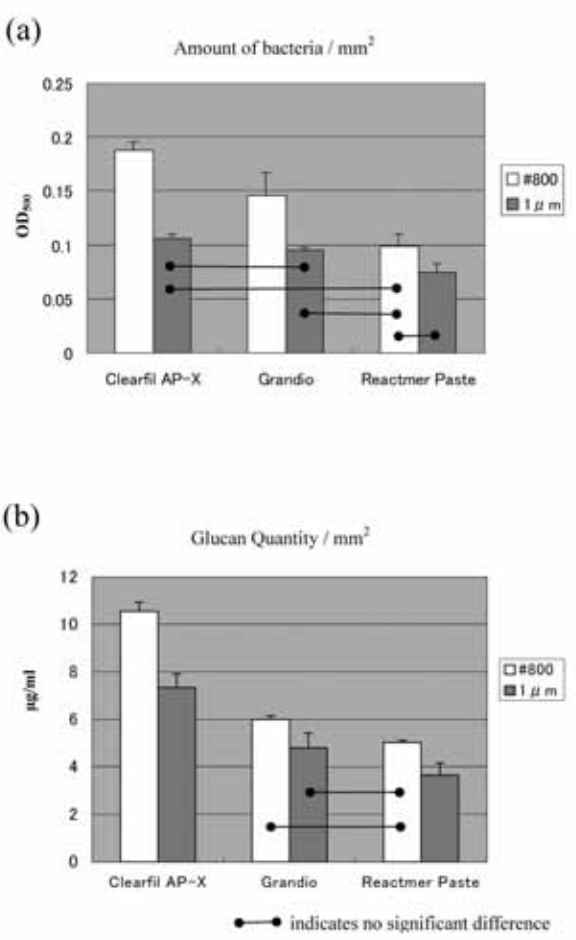

Fig. 2 Amount of bacteria (a) and glucan quantity (b) of the retained biofilms on the resin composites per mm^2 area are shown as mean ± standard deviation (n=4). Horizontal lines indicate no significant differences.

SEM observation of the biofilms at early stages

To observe initial biofilm attachment, the samples were observed by a scanning electron microscope (SEM). The biofilm was incubated for five hours in the AMS in the same manner described above. Samples were rinsed with PBS buffer and fixed in 4% paraformaldehyde with 1% glutaraldehyde in PBS for one hour. After which, they were rinsed with PBS three times for two minutes each, followed by rinsing with deionized water three times for two minutes each. They were then dehydrated through an ethanol series (50, 70, 80, 95, and 100%) for 15 minutes each, desiccated, and sputter-coated with 15 nm of gold using a SC-701AT (ELIONIX, Tokyo, Japan). Finally, specimens were examined using a SEM (JSM-5310LV, JEOL, Tokyo, Japan).

Bacterial viability test to determine the effects of residual monomer

LIVE/DEAD® BacLight™ Bacterial Viability Kit (Molecular Probes, Invitrogen, USA)[20] was used to

verify potential immediate effects on bacterial cells by unpolymerized residual monomers on the slab surfaces. *S. mutans* biofilms were formed on the resin slabs for one hour inside the AMS as described above. Immediately after removal from the AMS, the samples were washed with PBS, stained with BacLight™, and inspected using a fluorescence microscope (CKX41, Olympus, Tokyo, Japan).

In addition, approximately 55 mg of unpolymerized resin composite paste from each experimental material was incubated in 1 ml of Milli-Q water overnight at 37℃. The same bacterial suspension was then used for biofilm formation, and was aliquoted (1 ml) and pelleted. From the overnight incubation, 500 μl of Milli-Q water was added to the bacterial pellets separately. After thorough mixing, they were incubated for 10 minutes at RT, washed with PBS, followed by staining with BacLight™. These microscopic data represented the effects of unpolymerized monomer released from each uncured material paste on the biofilm.

Bis-GMA monomer (approximately 0.05 mg/ml) was also added to 500 μl of Milli-Q water, incubated with the bacteria for 10 minutes, and stained as above. This served as a control as well as freshly stained bacteria preserved in PBS.

SEM-EDS analysis of fluoride element on Reactmer slab surface

An energy dispersive X-ray spectroscope (EDS)[21], EMAX-7000 (Horiba, Japan), was used to determine the presence and release of elemental fluorine (F) on the surface of Reactmer slab before and after incubation for 20 hours in deionized water (Milli-Q water). Specimens were left to dry for 20 hours, desiccated, gold sputter-coated, and observed with a SEM S-4500 (Hitachi, Japan), followed by analysis of each surface by EDS. Aluminum (Al) and silica (Si) along with F were also measured on the line scan from secondary electron images of the Reactmer slab surfaces.

Statistical analysis

All numerical data were analyzed using the Statistical Package for Medical Science (SPSS Ver. 11 for Windows) for statistical procedures. Four specimens for surface roughness analysis and the measurement of biofilm attachment were analyzed for each group. The data for Ra values, amounts of bacteria/mm^2, and the levels of WIG/mm^2 were analyzed by two-way analysis of variance (ANOVA). The two factors analyzed were "material" and "polishing condition". Finally, a t-test was performed at a confidence level of 95%.

RESULTS

Surface roughness

Table 2 shows the surface roughness (Ra) values of the specimens. Two-way ANOVA revealed that there was no significant relationship between these two independent variables of material and polishing condition ($p>0.05$). The Ra values after treatment with SiC#800 were significantly higher than those of DP 1 μm ($p<0.05$). The three resin composites showed no significant differences among their Ra levels ($p>0.05$) when the polishing condition was the same, *i.e.*, either SiC#800 or DP 1 μm.

Biofilm formation on resin composite slabs

Single-species biofilms of sucrose-dependent *S. mutans* were formed on all resin composite surfaces, which appeared more condensed on SiC#800 samples than on DP 1 μm samples in all groups (Figs. 2a and b). On most occasions, the amounts of bacteria and glucan were highest on Clearfil AP-X SiC#800 compared to the other materials or surface conditions. Comparative differences were also observed in the amount of bacteria between the two surface conditions for all three materials: more on SiC#800 samples than on DP 1 μm samples. The differences were significant with Clearfil AP-X and Grandio, but not so with Reactmer Paste (Fig. 2a).

The quantity of water-insoluble glucan (WIG) in the artificial biofilms was influenced by both surface roughness (Ra) and material composition ($p<0.05$). Except for Grandio, WIG was more abundant in SiC#800 samples than in DP 1 μm samples. Interestingly, a significantly lower amount of WIG was recovered from Reactmer Paste DP 1 μm than from Reactmer Paste SiC#800 (Fig. 2b), whereas the difference was not significant for biofilm growth. In addition, significant difference in glucan quantity was detected between SiC#800- and DP 1 μm-polished Clearfil AP-X (Fig. 2b).

SEM observation

As observed by SEM, biofilm clusters (bacterial colonies) were less abundant in all DP 1 μm samples (Figs. 3b, d, and f) than in SiC#800 (Figs. 3a, c, and e). Moreover, in the former samples, there were

Table 2 Surface roughness values of the resin composites (Ra)

	SiC#800	DP 1 μm
Clearfil AP-X	2.22±0.13	0.25±0.66
Grandio	2.01±1.12	0.22±0.01
Reactmer Paste	2.15±0.16	0.23±0.01

Mean±SD (μm), Number of specimen was three for each group.

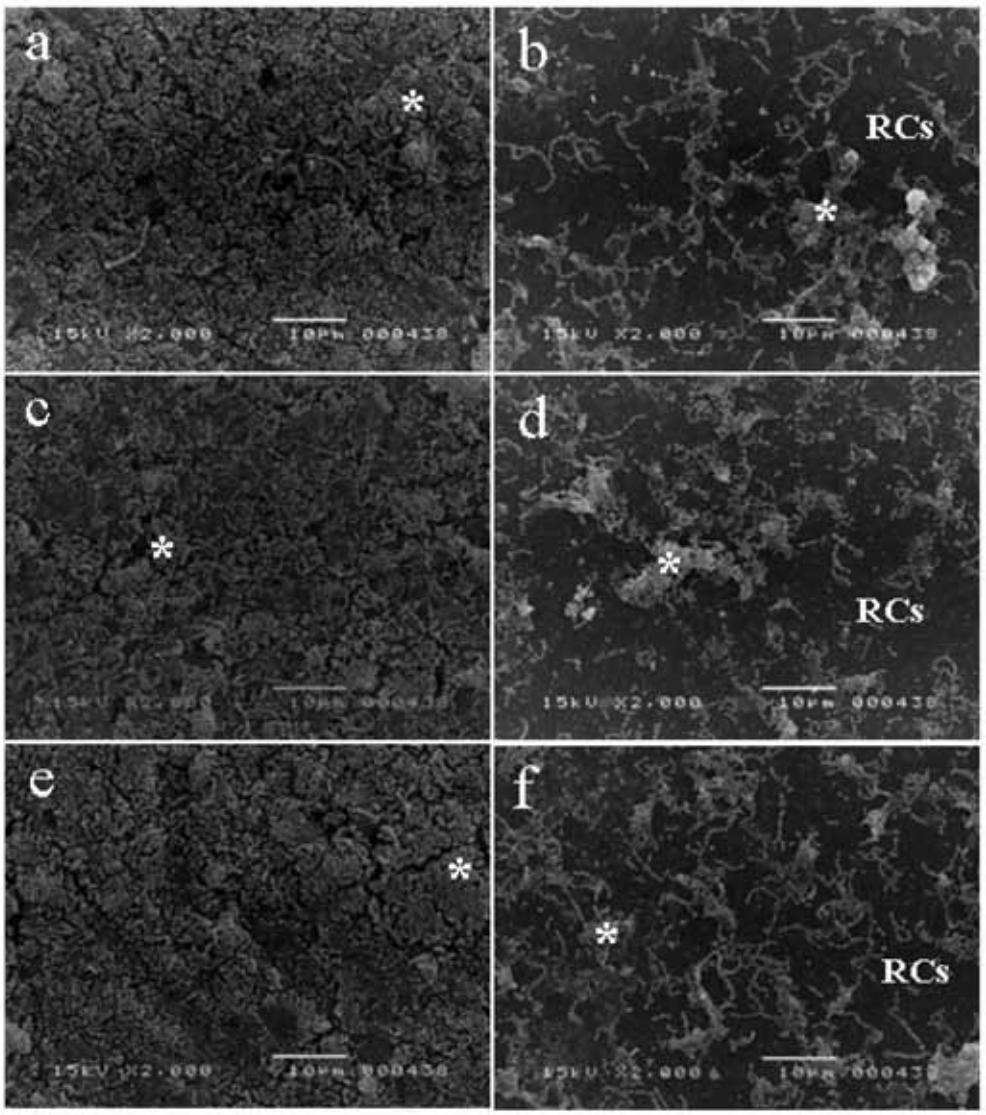

Fig. 3 SEM photomicrographs of biofilms on the resin composite surfaces after 5 hours (× 2,000). a: Clearfil AP-X SiC #800; b: Clearfil AP-X DP 1 μm; c: Grandio SiC #800; d: Grandio DP 1 μm; e: Reactmer Paste SiC #800; f: Reactmer Paste DP 1 μm. Asterisks (*) indicate "biofilm clusters".

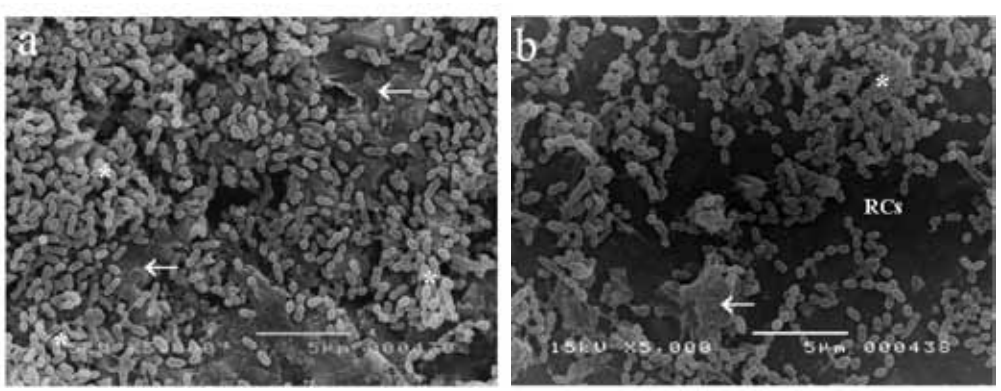

Fig. 4 SEM photomicrographs of biofilms on Clearfil AP-X surfaces after 5 hours (×5,000). a: SiC #800; b: DP 1 μm. **RCs** indicate surfaces of resin composites yet to be covered by biofilm; asterisks (*) indicate "biofilm clusters"; arrows (←) indicate "glucan surfaces".

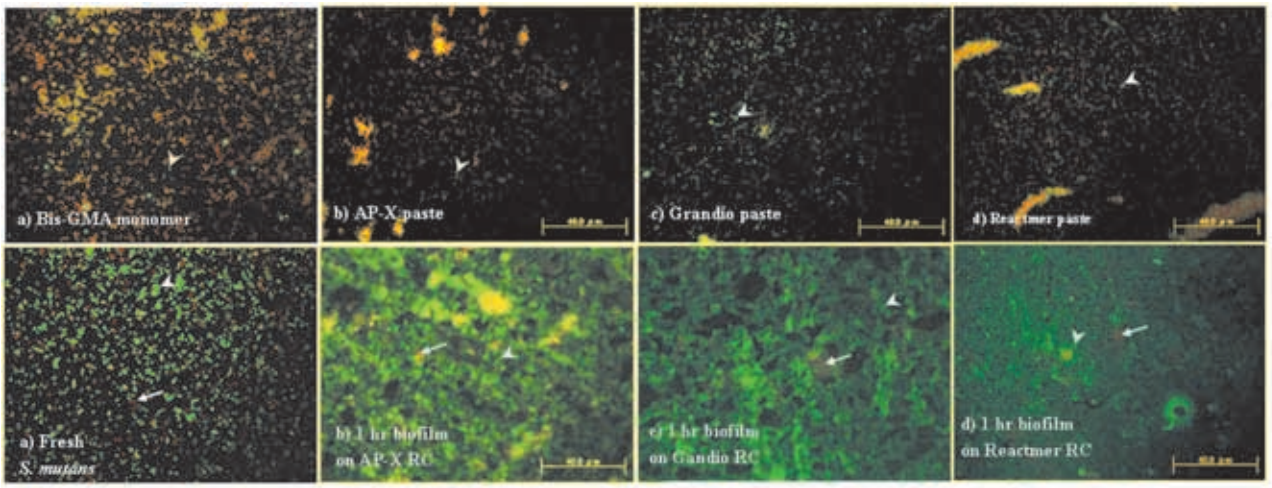

Fig. 5 Fluorescence photomicrography of *S. mutans* cells and biofilms stained with BacLight Bacterial Viability Kit. Arrowheads indicate **live** cells or clusters fluorescing **green**; arrows indicate **dead** cells or clusters fluorescing **red**.
Left column: (a) incubated with Bis-GMA monomer; (b) incubated in Milli-Q water with AP-X paste; (c) incubated in Milli-Q water with Grandio paste; (d) incubated in Milli-Q water with Reactmer paste.
Right column: (a) freshly stained *S. mutans* cells on side glass; (b)—(d) *S. mutans* biofilms on AP-X, Grandio, and Reactmer resin composite slabs.

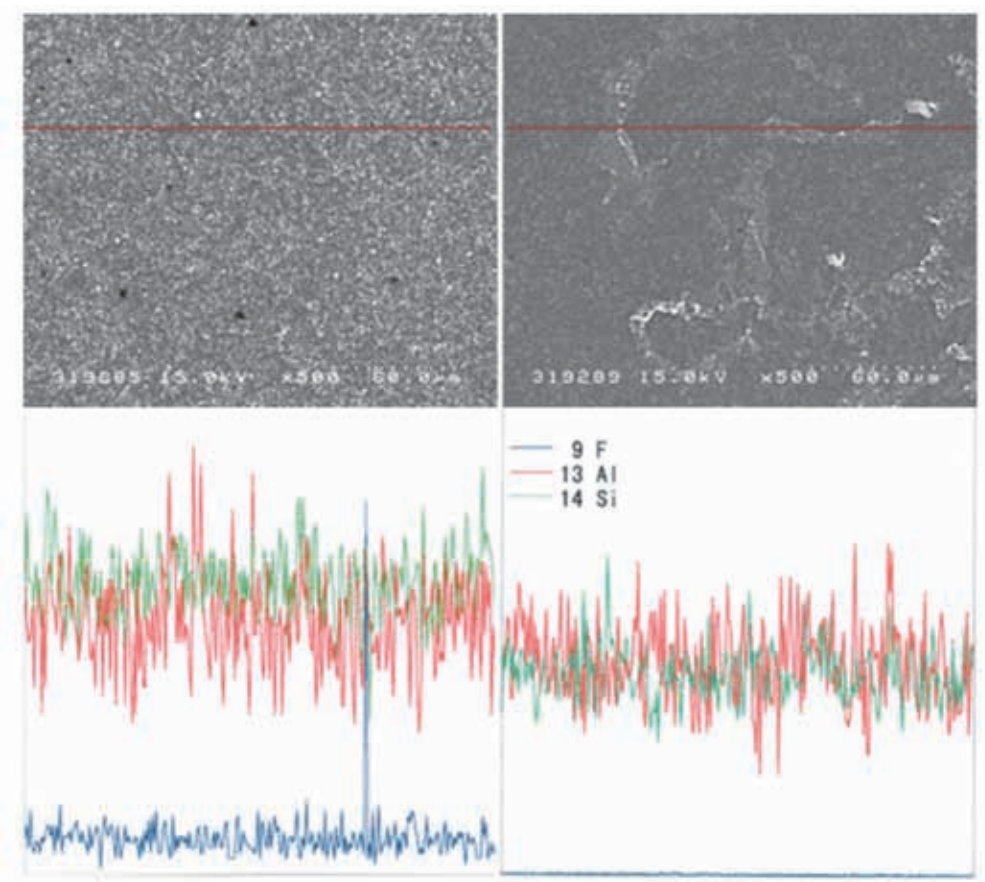

Fig. 6 Top: SEM photomicrographs representing Reactmer DP 1 μm surface before (left) and after (right) 20-hour incubation in Milli-Q water prior to EDS analysis. Bottom: Linear analysis of the area indicated by the horizontal line in the top photographs using EDS. Peaks of Al (red line), Si (green line), and F (blue line) indicate the distribution and relative quantity of each element on a Reactmer DP 1 μm slab surface. Small amount of F that was detected before incubation in Milli-Q, disappeared after 20 hours.

many streptococcal chains free of extracellular polysaccharide matrix — owing to WIG. Notably, there were free spaces in between the attached bacteria and growing biofilm on DP 1 μm surfaces, indicating that smooth surfaces were more resistant to bacterial adherence.

The Clearfil AP-X samples showed significant differences ($p<0.001$) as mentioned above (Figs. 2 and 4). As revealed by higher magnification, more bacteria and more glucan were clearly visible in Clearfil AP-X SiC#800 sample (Fig. 4a) than in Clearfil AP-X DP 1 μm sample (Fig. 4b). In the case of DP 1 μm samples generally, many parts of the resin composite surfaces were clearly not covered by biofilm after 5-hour incubation, which were indicated with 'RCs' in the figures (Figs. 3b, 3d, 3f, and 4b). Following inspection by SEM, it was clear that polishing by DP 1 μm improved the surface condition of the three resin composites in terms of resisting bacterial adherence and biofilm growth. After vortex agitation, retained biofilms on the samples in all groups after 20 hours appeared more or less the same topographically by SEM (data not shown).

Effects of residual monomer

BacLight™ staining data are shown in Fig. 5. Live *S. mutans* cells were visualized as green and dead cells as red in the same microscopic location of a biofilm on the slab surface, following a change of emission filters (insets). Clearly, more live cells were visible than dead cells in all experimental groups. Although the number of dead cells increased compared to fresh suspensions (control insets), detectable differences were not observed between the experimental groups. By contrast, the effects of the monomer released in Milli-Q water were evident for Clearfil AP-X, which was visualized as yellowish (experimental large) similar to that of Bis-GMA monomer (control large), although they were excited by blue light only. More live cells were visualized with Grandio or Reactmer Paste compared to Clearfil AP-X (experimental large). Further, there were no detectable differences between SiC#800 and DP 1 μm polishing conditions in all the three materials.

Fluorine on Reactmer slab surface

SEM-EDS data showed that elemental F was present on Reactmer slab surfaces when examined immediately after polishing, but which disappeared after 20-hour incubation in Milli-Q water (Fig. 6). EDS also indicated that elemental Al and Si were present at high concentrations and were also reduced after incubation in Milli-Q water.

DISCUSSION

In clinical situations, there exists a likelihood that the surface of restorative materials serves as a site for bacteria to adhere and initiate plaque formation, leading to secondary caries formation at the adjacent tooth structure. Against this background, it is important to investigate initial bacterial adhesion and biofilm formation on the surfaces of restorative materials.

S. mutans, which composes a significant proportion of the oral streptococci in caries lesions, has been identified as the major etiological agent of human dental caries. *S. mutans* is present in mature dental plaque associated with caries. Organic acids can be trapped within the glucan barrier produced by these bacteria, resulting in a prolonged low pH around the tooth surface[22].

Initial bacterial adherence to solid surfaces is facilitated by several factors, namely, electrostatic[23,24] and hydrodynamic interactions[25], thermodynamic parameters, specific binding mechanisms including adhesin-receptor interactions by which bacteria bind selectively to the surface as well as by attachment *via* polysaccharide matrix or glucans[26]. Adhesion to salivary pellicle-coated tooth surfaces is a critical step for oral bacterial colonization. Oral bacteria can adhere to the receptors of host origin in the salivary pellicle. However, the role of glucan-mediated interactions in *S. mutans* is primarily due to accumulation, following its sucrose (*i.e.*, glucan)-independent binding to saliva-coated tooth surfaces.

The AMS used in this study to grow *S. mutans* biofilms facilitates biofilm formation both under aerobic and anaerobic conditions with the temperature constant at 37℃. The reactor allows continuous recording of pH, and the reduction curves for pH were the same in all experiments: pH began to fall from 7.35 within two hours and was reduced to below 4.0 after 20 hours. Although the pH values recorded in this study might not accurately reflect the conditions of growing biofilms on resin composite surfaces and which did not allow differentiation between the surface properties, this system might be a reliable model for similar future studies. In this regard, attempts have been made to analyze the pH at the interface of biofilms and their host substrates as well as in and around the caries lesions[27].

Components of the AMS device — which can become contaminated — can be autoclaved. With this advantageous feature, biofilms grown inside the AMS in this study were consistent and reliable like several other *in vitro* model systems. The EYELA EPC-2000 computer control of the AMS, perhaps the first of this type which could produce biofilms of different structures by altering the flow rates within a single experiment, might also prove useful in future studies. Most importantly, this system facilitated biofilm adherence studies in relation to the surface properties of different dental materials under similar

microbial culture conditions close to that of the oral environment. By using the AMS to compare the three resin composite materials in the present study, a study model was hence established to provide a better understanding of how the surface properties of a dental material would influence biofilm adherence and growth.

After 20 hours of incubation, relatively mature biofilms were formed on the sample surfaces. As a result, measurable amounts of retained biofilm were still obtained after strong vortex agitation. However, 20-hour, post-agitation surface conditions were not clear enough to detect the topographical differences visually by SEM (data not shown). Nevertheless, SEM photomicrographs of 5-hour biofilms were presented in this report to visualize the topographical differences more clearly on three different materials with two different surface conditions.

Dental plaque adheres better and accumulates more quickly on rough surfaces *in vivo*. In the present study, there were free spaces in between attached bacteria and growing biofilm on DP 1 μm surfaces, indicating that smooth surfaces (*i.e.*, low Ra value) were important in modulating biofilm adherence and growth. These findings indicated that polishing a composite surface was very important in hindering or slowing down biofilm adherence and growth.

Fillers and matrices of dental resin composites also influence the growth of bacterial biofilms. However, the amount of biofilm accumulation varies according to the particle size of fillers and monomer components of the resin matrix[10,19,28,29]. In the present study, the effects of residual matrix monomer on bacterial cells were not evident. Data of BacLight™ Viability Kit clearly demonstrated that most *S. mutans* cells died within 10 minutes of incubation time by the monomers that instantly dissolved into the bacterial suspension from the uncured material pastes. However, proportionally, more live cells could be detected at even one hour after the AMS was started. Therefore, a time interval of one hour was required at the beginning to obtain a detectable number of bacteria to adhere on the surfaces of the materials well before biofilms began to mature. All three resin composites reacted comparably with *S. mutans* cells in the biofilms. Apparently, there were no remarkable changes in bacterial viability. Quite a large number of bacteria remained alive and might form biofilms actively even in the presence of trace amounts of residual matrix monomer.

Surface roughness also influences adhesion on enamel, probably because of the greater surface area provided and the provision of protected sites for colonization. Results of the present study demonstrated that improvement in surface conditions to resist biofilm adherence could be achieved if resin composites were carefully polished. The improvement patterns, however, were not similar. The greatest improvement was witnessed in Clearfil AP-X and the least in Reactmer Paste.

These results must be due to differences in material composition, and whereby filler loading playing a dominant role. Clearfil AP-X contained about 85% barium glass and silica fillers, whereby these filler types might have provided less protected sites for bacterial colonization than the fillers of the other two materials. In other words, highly-loaded filler particles might be sites capable of resisting bacterial colonization. Peradventure, polishing made the larger-sized barium glass and silica fillers more slippery, thereby improving AP-X surface property in terms of wettability and self-cleaning[30]. Grandio, on the other hand, contained about 56% nanofillers of barium aluminum silicate, and which showed significant improvement in resisting biofilm formation. In contrast, Reactmer Paste contained a novel filler material known as fully pre-reacted glass polyalkenoate fillers (F-PRG)[31] as well as ultra fine fillers totaling 78%. Although the influence of polishing was manifested marginally, no significant differences in bacteria amount were observed. Filler size of the latter two materials was relatively small — which probably explained why polishing did not render the surfaces biofilm-resistant as with Clearfil AP-X.

Polishing the surfaces of resin composites is one important process for direct resin composite restorations. In the clinical setting, the polishing process should entail the consideration of resisting biofilm adherence and growth around the composite restoration. Selection of instruments for polishing is another factor influencing biofilm adherence in the oral environment. Refurbishment of composite restorations is also very important at patient recall so as to control the surface texture of the restorations.

Fewer cariogenic microorganisms were found in plaque adjacent to orthodontic brackets retained with a GIC compared to resin composites. One possible reason for the antibacterial activity of GIC is thought to be due to fluoride[32,33]. GIC is effective in inhibiting bacterial growth and adhesion to solid surfaces such as restorations and teeth. In the present study, biofilm growth was somewhat affected by chemical composition. Reactmer Paste, which contains releasable fluoride and F-PRG filler material[34], showed remarkable resistance even in the SiC#800 group. The presence of elemental fluorine on Reactmer Paste slab surface was another potent indication of fluoride release as detected by SEM-EDS. Incubation in Milli-Q water for 20 hours might have dissolved most of the releasable fluoride.

Similarly, released fluoride dissolved in liquid and perhaps interfered with the process of WIG formation or the maturation of biofilms formed by *S. mutans*. This suggestion was proffered because it has been shown previously that WIG production in *S. mutans* biofilms was reduced by only 250 ppm of fluoride[35].

CONCLUSIONS

Results of this study indicated that polishing and composition of the direct resin composites strongly influenced bacterial adherence, but did not show similar potency in resisting biofilm formation. Therefore, it was concluded that appropriately performed polishing could render certain resin composite surfaces more resistant to bacterial adherence than others. In other words, polishing did not cause all resin composites to be similarly resistant to biofilm formation. The water insoluble glucans in *S. mutans* biofilms were also somewhat affected by the chemical composition of the fluoride-releasing resin composite used in this study, probably due to the effect of released fluoride.

ACKNOWLEDGEMENTS

This study was supported by the Center of Excellence (COE) Program for Frontier Research on Molecular Destruction and Reconstruction of Tooth and Bone at Tokyo Medical and Dental University, Tokyo, Japan, and a Grant-in-Aid from the Japan Society for the Promotion of Science (JSPS, No. 16390544). In addition, the authors would like to express their gratitude to Dr. Howard H. Kuramitsu, State University of New York at Buffalo, for his kind contributions in editing the manuscript.

REFERENCES

1) Rueggeberg FA, Ergle JW, Lockwood PE. Effect of photoinitiator level on properties of a light-cured and post-cure heated model resin system. Dent Mater 1997; 13: 360-364.
2) Ferracane JL, Berge HX, Condon JR. *In vitro* aging of dental composites in water — Effect of degree of conversion, filler volume, and filler/matrix coupling. J Biomed Mater Res 1998; 42: 465-472.
3) Park C, Robertson RE. Mechanical properties of resin composites with filler particles aligned by an electric field. Dent Mater 1998; 14: 385-393.
4) Li Y, Swartz ML, Phillips RW, Moore BK, Roberts TA. Effect of filler content and size on properties of composites. J Dent Res 1985; 64: 1396-1401.
5) Braem M, Finger W, Van Doren VE, Lambrechts P, Vanherle G. Mechanical properties and filler fraction of dental composites. Dent Mater 1989; 5: 346-349.
6) Svanberg M, Mjor IA, Orstavik D. Mutans streptococci in plaque from margins of amalgam, composite, and glass-ionomer restorations. J Dent Res 1990; 69: 861-864.
7) Barsotti O, Morrier JJ, Rocca JP, Duprez JP. Initial colonization of composite resins by *Streptococcus mutans*: the influence of polishing. Dumont Rev Odontostomatol 1989; 18: 213-220.
8) Carlen A, Nikdel K, Wennerberg A, Holmberg K, Olsson J. Surface characteristics and *in vitro* biofilm formation on glass ionomer and composite resin. Biomaterials 2001; 22: 481-487.
9) Ikeda M, Nikaido T, Foxton RM, Tagami J. Shear bond strengths of indirect resin composites to hybrid ceramic. Dent Mater J 2005; 24: 238-243.
10) Imazato S, Ebi N, Takahashi Y, Kaneko T, Ebisu S, Russell RR. Antibacterial activity of bactericide-immobilized filler for resin-based restoratives. Biomaterials 2003; 24: 3605-3609.
11) Kawai K, Torii M, Tsuchitani Y. Effect of resin components on the growth of Streptococcus mutans. J Osaka Univ Dent Sch 1988; 28: 161-170.
12) Kolenbrander PE, London J. Adhere today, here tomorrow: oral bacterial adherence. J Bacteriol 1993; 175: 3247-3252.
13) Konishi N, Torii Y, Kurosaki A, Takatsuka T, Itota T, Yoshiyama M. Confocal laser scanning microscopic analysis of early plaque formed on resin composite and human enamel. J Oral Rehabil 2003; 30: 790-795.
14) Lie T. Morphologic studies on dental plaque formation. Acta Odontol Scand 1979; 37: 73-85.
15) Kolenbrander PE, Andersen RN, Blehert DS, England PG, Foster JS, Palmer RJ Jr. Communication among oral bacteria. Microbiol Mol Biol Rev 2002; 66: 486-505.
16) Scheie AA, Petersen FC. The biofilm concept: Consequences for future prophylaxis of oral diseases? Crit Rev Oral Biol Med 2004; 15: 4-12.
17) Hansel C, Leyhausen G, Mai UE, Geurtsen W. Effects of various resin composite (co)monomers and extracts on two caries associated micro-organisms *in vitro*. J Dent Res 1998; 77: 60-67.
18) Yamamoto K, Ohashi S, Taki E, Hirata K. Adherence of oral streptococci to composite resin of varying surface roughness. Dent Mater J 1996; 15: 201-204.
19) Ashwell, G. The phenol-sulfuric acid reaction for carbohydrates. Methods Enzymol 1966; 8: 93-95.
20) Freese HM, Karsten U, Schumann R. Bacterial abundance, activity, and viability in the eutrophic River Warnow, northeast Germany. Microb Ecol 2006; 51: 117-127.
21) Ichinose S, Muneta T, Sekiya I, Itoh S, Aoki H, Tagami M. The study of metal ion release and cytotoxicity in Co-Cr-Mo and Ti-Al-V alloy in total knee prosthesis — scanning electron microscopic observation. J Mater Sci Mater Med 2003; 14: 79-86.
22) Van Houte J, Russo J, Prostak KS. Increased pH-lowering ability of Streptococcus mutans cell masses associated with extracellular glucan-rich matrix material and the mechanisms involved. J Dent Res 1989; 68: 451-459.
23) Poortinga AT, Bos R, Busscher HJ. Electrostatic interactions in the adhesion of an ion-penetrable and ion-impenetrable bacterial strain to glass. Colloids Surf B Biointerfaces 2001; 20: 105-117.
24) Poortinga AT, Smit J, van der Mei HC, Busscher HJ. Electric field induced desorption of bacteria from a

conditioning film covered substratum. Biotechnol Bioeng 2001; 76: 395-399.
25) Weerkamp AH, Uyen HM, Busscher HJ. Effect of zeta potential and surface energy on bacterial adhesion to uncoated and saliva-coated human enamel and dentin. J Dent Res 2001; 67: 1483-1487.
26) Hanada N, Kuramitsu HK. Isolation and characterization of the Streptococcus mutans gtfC gene, coding for synthesis of both soluble and insoluble glucans. Infect Immun 1988; 56: 1999-2005.
27) Nikaido T, Moriya K, Hiraishi N, Ikeda M, Kitasako Y, Foxton RM, Tagami J. Surface analysis of dentinal caries in primary teeth using a pH-imaging microscope. Dent Mater J 2004; 4: 621-627.
28) Takahashi Y, Imazato S, Russell RR, Noiri Y, Ebisu S. Influence of resin monomers on growth of oral streptococci. J Dent Res 2004; 83: 302-306.
29) Hashimoto K, Yamamoto H. Influence of composite resin components on adherence of oral streptococci. Jpn J Conservative Dent 1998; 41: 1-15.
30) Blossey R. Self-cleaning surfaces — virtual realities. Nat Mater 2003; 2: 301-306.
31) Tay FR, Sano H, Tagami J, Hashimoto M, Moulding KM, Yiu C, Pashley DH. Ultrastructural study of a glass ionomer-based, all-in-one adhesive. J Dent 2001; 29: 489-498.
32) Kawai K, Takaoka T. Inhibition of bacterial and glucan adherence to various light-cured fluoride-releasing restorative materials. J Dent 2001; 29: 119-122.
33) Wu SS, Yap AUJ, Chelvan S, Tan ESF. Effect of prophylaxis regimens on surface roughness of glass ionomer cements. Oper Dent 2005; 3: 180-184.
34) Itota T, Nakabo S, Iwai Y, Konishi N, Nagamine M, Torii Y. Inhibition of artificial secondary caries by fluoride-releasing adhesives on root dentin. J Oral Rehabil 2002; 29: 523-527.
35) Koo H, Schobel B, Scott-Anne K, Watson G, Bowen WH, Cury JA, Rosalen PL, Park YK. Apigenin and tt-farnesol with fluoride effects on *S. mutans* biofilms and dental caries. J Dent Res 2005; 84: 1016-1020.