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Biofilm formation affects surface properties of novel bioactive glass-containing composites

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Abstract

Objectives—This study investigated the effects of bacterial biofilm on the surface properties of novel bioactive glass (BAG)-containing composites of different initial surface roughness.

Methods—BAG (65 mole% Si; 4% P; 31% Ca) and BAG-F (61% Si; 31% Ca; 4% P; 3% F; 1% B) were synthesized by the sol-gel method and micronized (size ~0.1–10 μ m). Composites with 72 wt% total filler load were prepared by replacing 15% of the silanized Sr glass with BAG, BAG-F, or silanized silica. Specimens (n=10/group) were light-cured and divided into 4 subgroups of different surface roughness by wet polishing with 600 and then up to 1200, 2400, or 4000 grit SiC. Surface roughness (SR), gloss, and Knoop microhardness were measured before and after incubating in media with or without a *S. mutans* (UA 159) biofilm for 2 wks. Results were analyzed with ANOVA/Tukey's test ($\alpha = 0.05$).

Results—The SR of the BAG-containing composites with the smoothest surfaces (2400/4000 grit) increased in media or bacteria; the SR of the roughest composites (600 grit) decreased. The gloss of the smoothest BAG-containing composites decreased in bacteria and media-only, but more in media-alone. The microhardness of all of the composites decreased with exposure to media or bacteria, with BAG-containing composites affected more than the control.

Significance—Exposure to bacterial biofilm and its media produced enhanced roughness and reduced gloss and surface microhardness of highly polished dental composites containing a bioactive glass additive, which could affect further biofilm formation, as well as the esthetics, of restorations made from such a material.

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1. Introduction

Bioactive glasses (BAGs) are represented by a range of dense, amorphous calcium, sodium phosphosilicate materials that can develop strong chemical bonds with the collagen in living tissues [1]. This characteristic is known to be related to their ability to form a biologically active hydroxycarbonate apatite layer on their surfaces by releasing ions rapidly when exposed to an aqueous medium, such as body fluids [2,3]. Thus, BAGs are considered as promising materials for dental use because of their biocompatibility and capability for stimulating the regeneration of hard tissues. Recently, BAG has been considered as a strategic additive for dental restorative materials because it has been shown to possess antimicrobial properties as well as the capability for releasing ions needed for tooth remineralization [4,5]. While it has been shown that BAG has a high elastic modulus and a low fracture toughness compared to more conventional fillers, such as a strontium glass [6], a recent study showed that BAG containing composites demonstrate adequate and stable mechanical properties, comparable to three successful commercial composites [4]. Also, when fluoride-containing BAG (BAG-F) was added to a composite formulation, the composite had the potential to release both calcium and fluoride ions in aqueous solution and could also be readily recharged with fluoride [7].

It is desirable for dental composite materials to have high surface hardness, and to be polishable to a very low surface roughness and to maintain these characteristics during service. Recent studies of resin composite showed that the surface roughness was negatively correlated with the surface microhardness, and that finishing/polishing procedures resulted in harder and smoother surfaces [8,9]. A study using water and acidic solutions as storage media showed that both the surface roughness and microhardness of resin composites decreased after five weeks [10]. It has also been reported that *S. mutans* biofilms grown on the surface of resin composite increased surface roughness without influencing surface microhardness, but that the change in surface integrity could cause a further accumulation of biofilm [11]. These studies suggest that the surface properties of dental composites may be affected by storage media and bacterial exposure, but that more systematic studies are needed. In particular, it is important to systematically study the effect that surface roughness has on biofilm formation on composites, as it has been suggested that biofilm formation is enhanced when average surface roughness (Ra) exceeds 0.2 μm [12].

In the present study, novel composites containing bioactive glass fillers, with and without fluoride, were synthesized and characterized in terms of surface roughness, composition, and effects of *S. mutans* colonization. The aim of this study was to systematically evaluate the effect of surface roughness (less than and greater than 0.2 μm) of the BAG and BAG-F composites on biofilm formation and to investigate how biofilm accumulation can affect the surface properties of the composites.

2. Materials and methods

2.1. Preparation of BAG

BAGs used in this study were synthesized in our laboratory by sol-gel methods as previously described [5]. After the solution had gelled and was cooled rapidly, the synthetic

glasses were ball milled in ethanol and sieved to a gross particle dimension of less than 38 μm . The particles were then further processed using a Micronizer Jet Mill (Sturtevant Inc., Hanover, MA, USA), produce a fine particle size (0.04 – 3.0 μm) as determined by laser particle size measurements (Beckman Coulter LS13 320, Brea, CA, USA).

2.2. Formulation of experimental composites

The three experimental composites all contained 57 wt% of silane treated strontium glass filler (1–3 μm average size, Bisco, Inc.), and were further modified as follows: the control group included a silane-treated aerosil-silica (OX-50, Degussa) as a control, while micronized BAG (BAG65) and fluorine-contained micronized BAG (BAG61) replaced the silica in the BAG and BAG-F groups (Table 1). The composites were produced using a centrifugal mixing device (Speed-Mixer DAC 150 FVZ, Hauschild, Germany) for 2 minutes at 2400 rpm and stored in sealed containers at room temperature before testing.

2.3. Specimen preparation

Forty-eight disk-shaped specimens of each group (10 mm diameter by 2 mm thickness) were prepared in rubber molds with both the top and bottom surface covered by microscope slide glasses to extrude excess resin. All materials were light-cured using a curing unit with a 12 mm diameter light guide (Demi™, Kerr, Orange, CA, USA) for 40 seconds at 520–580 mW/cm^2 on each side. Specimens were separated from the mold, any flash on the lateral border was carefully removed, and the specimens were aged for 24 hours in the dark at ambient humidity. To produce a reproducible and consistent surface roughness, a specimen holder was created to hold the specimen firmly while delivering a gentle axial force perpendicular to the surface of the silicon carbide paper rotating on a polishing wheel (Struers inc., Cleveland, OH, USA) under cooling water. Every group was divided into four polishing subgroups of twelve specimens designed to produce four distinct ranges of surface roughness. The roughness targets were established by a pilot test using 44 composite specimens; Subgroup P600 was polished against SiC paper of 600-grit; P1200 against 600- and 1200-grit sequentially; P2400 against 600-, 1200-, and 2400-grit sequentially; and P4000 against 600-, 1200-, 2400-, and 4000-grit sequentially. Every polishing step was performed by one investigator checking repeatedly during the polishing to ensure that the correct range of target average surface roughness (Ra) values were obtained.

2.4. Baseline properties testing

The baseline surface roughness (Ra, μm) of each specimen was measured using a surface roughness tester TR200 (TIME Group, Pittsburgh, PA, USA) with a tracing length of 5 mm and a cutoff value of 0.25 μm to maximize filtration of surface waviness. Gloss was measured using a small-area glossmeter (Novo-Curve, Rhopoint Instrumentation, East Sussex, UK), with a square measurement area of 2×2 mm and 60° geometry. Gloss measurements were expressed in gloss units (GU). Both were measured at four different positions on the specimen, rotated clockwise, at a right angle. Three Knoop indentations were made in each specimen using a universal hardness tester (Duramin, Struers, Ballerup, Denmark) with a load of 0.98 N applied for 5 s (Knoop hardness number, KHN). One specimen of each subgroup ($n = 12$) was randomly assigned to be examined using a scanning electron microscope (SEM). The specimen was sputter coated with gold-palladium

in a Denton Vacuum Desk II (Denton Vacuum Inc., Moorestown, NJ, USA) and the surface was observed at 15.0 kV in an SEM under 2000× magnification (Quanta 200, FEI Company, Hillsboro, OR, USA).

2.5. Biofilm procedure

Overnight cultures of *S. mutans* (strain UA159) grown in brain heart infusion (BHI) at 37°C in an incubator with 5% CO₂ were measured for optical density at 600 nm (OD₆₀₀) and then diluted to an OD₆₀₀ of 0.4 – 0.6. A 1:10 dilution of the stock solution in new BHI medium was then incubated for 3 hours to obtain OD₆₀₀ = 0.3, which represents a bacterial concentration of 9×10^7 CFU/mL based on previous calibration studies. Culture media was trypticase soy broth (BBL™ Trypticase™ Soy Broth, BD diagnostics, MD, USA) with 3 wt % sucrose (Fischer Science Education, Hanover Park, IL, USA). Six specimens from each group were chosen for the biofilm group and were inoculated with *S. mutans*. Five specimens were used for the group aged in media only. All specimens (n = 132) were sterilized using 70% ethyl alcohol in an ultrasonic bath for 15 minutes followed by absolute ethyl alcohol for 15 minutes. The sample disks were placed at the bottom of the wells of six well culture plates. For the *S. mutans* group, a 1:100 subculture of *S. mutans* was added to the culture media and 5 mL was placed in each well using a sterile pipette. For the control group, only 5 mL of the culture media free of *S. mutans* was placed in each well. All specimens were incubated in 5% CO₂ at 37°C for 14 days, with the culture media being removed every 24 hour and replaced with 5mL of fresh culture media using a sterile pipette. On the 13th day of the experiment, the samples were checked for contamination by culturing in BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (BD diagnostics, Hunt Valley, MD, USA).

One of the samples in each *S. mutans* group (n = 12) was chosen for SEM examination of the biofilm by fixing with 4% glutaraldehyde in distilled water at room temperature. The biofilms were removed from the other samples by gently wiping with tissues (Kimwipes™, Kimberly-Clark, Dallas, TX, USA) and sterile water in order to prepare the specimens' surface for surface roughness, gloss, microhardness, and SEM imaging.

2.6. Post incubation properties

The samples on which biofilm was formed were carefully removed from the fixative solution and were dried at room temperature for 24 hours. They were sputter coated with gold-palladium, and their surfaces were examined under SEM in the same way as the pretreatment examination. In all the other samples of which biofilm was removed from the specimen surface, Ra, GU, and KHN measurements were repeated in the same way as the baseline measurements. They were also coated and studied using SEM.

2.7. Statistical analysis

Statistical comparisons between pre- and post-treatment were made among each polishing level of P600, P1200, P2400, and P4000 for all three materials. The normality of the studied parameters was checked using the Kolmogorov-Smirnov test. If all followed normal distribution, the values of the studied parameters in each group were compared by paired t-test. Otherwise, they were analyzed using Wilcoxon Signed Ranks Test. For within-group

comparison, if they satisfied the assumptions of normality and homogeneity of variance (Levene's test), they were compared by analysis of variance (ANOVA). According to the results of Levene statistic, post hoc comparisons were performed using Tukey or Dunnett's T3. If any of the parameters did not follow a normal distribution, they were analyzed using the Kruskal Wallis test. All statistical analyses were performed with a significance level of $p = 0.05$ using SPSS 21 (IBM Corp., Somers, NY, USA).

3. Results

3.1. Surface roughness between pre- and post-treatment

There were no significant differences in the initial surface roughness of the three different types of composites (Table 2). For the control composite, the Ra value of P600 showed a significant decrease when aged in media-only, while the Ra of P600, P1200, and P2400 showed a significant decrease when aged with the biofilm. For the BAG-containing composite group, the Ra of P600 showed a significant decrease in both media-only and biofilm. But Ra values of all of the other polishing groups showed a significant increase in the media-only, while the Ra of P2400, P4000 showed a significant increase in the *S. mutans* group ($P < 0.05$). For the BAG-F group, the Ra of P1200, P2400, P4000 showed a significant increase in the media-only, while the Ra of P600 showed a significant decrease. The Ra values of P1200, P2400, and P4000 showed a significant increase in the *S. mutans* group.

3.2. Surface gloss between pre- and post-treatment

Specimens polished on 600 and 1200 grit were basically devoid of gloss ($GU < 3$) (Fig. 1). For the control composite, the GU of P600 showed a significant increase in the media-only group, while GU of P600 and P1200 showed a significant increase and the GU of P4000 showed a significant decrease in the *S. mutans* group ($P < 0.05$). For the BAG group, the GU of P1200, P2400, P4000 showed a significant decrease in the media-only, while the GU of P600 and P1200 showed a significant increase and the GU of P2400 and P4000 showed a significant decrease in the *S. mutans* group. For the BAG-F group, the GU of P600 showed a significant increase and the GU of P2400 and P4000 showed a significant decrease in the control group, while the GU of P600 and P1200 showed a significant increase and the GU of P2400 and P4000 showed a significant decrease in the *S. mutans* group.

3.3. Surface microhardness between pre- and post-treatment

Initial KHN values generally increased with increasing level of polishing for each composite (Fig. 2). The BAG-F group showed a significantly lower value of KHN compared to the control and BAG groups for P600 before treatment, while there was a significant decrease between the groups ($A > B > C$) after treatment with or without the biofilm. There were no significant differences in KHN between the groups of P4000 before treatment, while there was a significant decrease between the groups ($A > B > C$) after treatment regardless of the inoculation of *S. mutans* ($P < 0.05$). However, all KHN showed significant decreases between pre- and post-treatment regardless of whether the composites were exposed to media-only or biofilm.

3.4. Change of surface roughness, gloss and microhardness – correlation

Each Ra value measured before and after *S. mutans* biofilm formation was significantly decreased as the level of polishing increased, as shown by a high negative correlation, while each gloss value and surface microhardness before and after the biofilm formation was significantly increased with an increasing level of polishing, as shown by a high positive correlation (Table 3).

3.5. Comparison of microscopic images

Thicker and more clustered biofilms were identified on the rougher surfaces, while thinner and more dispersed bacterial colonies were observed on the smoother surfaces by SEM. A thin biofilm with more sparse clusters was found with a higher level of polishing in every group. Also, less clusters formed in the biofilm were observed for the BAG and BAG-F groups compared to the control group at the same level of polishing especially with highly polished composites (Fig. 3–5 d, h, l, p).

The initial surfaces of all samples at the same polishing level showed similarity irrespective of type of composite (Fig. 3–5 a, e, i, m). The overall integrity of the filler-resin interface in the control group was retained, while more small voids caused by dissolution of the BAG fillers in BAG containing composites were observed after storage both in media-only and in *S. mutans* culture. The surface of BAG-F group showed more voids than that for the BAG group at the same polishing level and treatment. The surface of BAG and BAG-F groups stored in the media-only showed more voids than the surfaces stored with the biofilm at the higher level of polishing, which made the surface rougher (Fig. 3–5, Table 2).

4. Discussion

The composites evaluated in this study showed variable changes in surface roughness, gloss and hardness as a result of aging in media-only or bacterial biofilm. The control resin composite showed a significant decrease in post-treatment surface roughness at every level of polishing under the influence of *S. mutans* biofilm, while showing little difference after storing in the media-only. This “polishing effect” producing lower roughness upon exposure to a biofilm is at odds with previous studies demonstrating that *S. mutans* growth on resin composite resulted in an increased surface roughness, which was suggested to be caused by the bacteria-material interaction and the consequent partial surface degradation [11]. In another study investigating the influence of *S. mutans* biofilm on the surface of four restorative materials, including nano-filled resin composite, surface roughness increased after being subjected to a biofilm for 30 days compared to aging in media-only, which was unaffected. The authors explained that Bis-EMA used in the material was more resistant to biodegradation due to the increased hydrophobicity of the monomer compared to Bis-GMA [13]. The difference in outcomes may be related to the initial surface, which in many studies is smooth and resin-rich by virtue of being produced by curing against a matrix material, without subsequent polishing. In the present study, bacteria forming on a rough surface and producing degradation of the resin matrix may actually remove some of the surface material, leaving the surface less rough and uneven.

Compared to the control composite, the post-treatment surface roughness of BAG and BAG-F composites showed a decrease when polished to a lesser level and an increase when having an initially higher polishing level, irrespective of biofilm formation. A previous study reported a positive correlation between surface roughness of restorative materials, including indirect and direct composite resins, and the adhesion of vital *S. mutans* [14]. In a review of the literature of the effect of initial surface roughness of oral hard materials on increased plaque accumulation, a threshold surface roughness for bacteria retention in vivo was suggested to be at an Ra above 0.2 μm [12]. In the current study, the Ra of specimens polished with P2400 and P4000 were far below the threshold level of 0.2 μm . The SEM analysis showed that all composites formed dense biofilms by 14 days, independent of level of polish. However, there was less cell aggregation and a more sparse cell density observed on BAG and BAG-F composites as compared with the control when the surfaces were initially polished to a higher level. Exposure of BAG and BAG-F composite to any aqueous solution results in particle dissolution that alters surface integrity, as shown by the SEM results. Thus, the more highly polished surfaces roughen with exposure time, making them more hospitable for bacterial adhesion. However, as the biofilm forms, it is likely that the composite surface below the biofilm may be “protected” to some extent by the cells, and no longer exposed to the media, which may be becoming acidic as a result of the bacterial activity. Due to the “patchy” nature of biofilms, parts of the surface will be covered by cells while others will be left exposed to the media. Therefore, BAG and BAG-F particles on the surface may remain more exposed to the aqueous environment in the media-only than in the biofilm-coated area, contributing to the increase of surface roughness that is attributed more to the dissolution of the BAG fillers than by bacterial degradation of the exposed resin surface. This is consistent with the result of higher post-treatment roughness for BAG and BAG-F composites with higher initial polish when aged in the media-only compared to the *S. mutans* biofilm. It should be noted that the experiment conducted in this study was representative of a static situation, where the bacteria were not exposed to a flowing environment when trying to adhere to and colonize the composite surfaces. Thus, this may be considered a best case scenario for biofilm formation.

Sol-gel derived BAG has the potential to release calcium ions, causing an elevation in local pH of the interfacial solution, which can contribute to an antibacterial effect [7,15]. Fluoride is also known to have the ability to act as a buffer to neutralize acids produced by bacteria and to suppress the growth of cariogenic bacteria by reducing their aciduricity and acidogenicity [16,17]. However, the potential for the calcium or fluoride release to reduce biofilm formation may be unrealized unless the effective level of ions is high enough [18]. In this experiment, the SEM analysis showed evidence for the erosion or deterioration of the BAG particles, likely due to ion release from their surface, which correlated with a reduction of bacterial aggregation on the surface of BAG and BAG-F composites compared to that of the control composite. This suggests that either the calcium released, or the fluoride released from the BAG-F composite, or both, had some local influence on the biofilm formation. The effect overall was not great however, as the eventual biofilm was fairly thick on all composites at 14 days.

Gloss is closely related to the light reflectance influenced by microstructural characteristics of the material surface [19]. It has been reported that there is a significant inverse linear correlation between the average roughness and the gloss [20]. However, even materials with similar surface roughness values can show very different surface characteristics, as verified with surface profile plots or SEM [21]. Also, the mean size, shape, and index of refraction of the filler, and the homogeneity of the filler-matrix complex can affect the surface microstructure, which can allow materials of similar roughness to show different gloss [22]. In this study, there were significant differences in the initial GU between composite groups with similar initial surface roughness, likely due to the fact that the composites had different fillers with different chemical, physical and optical properties in each group. T

The post-treatment GU for every composite increased for P600 and P1200 with biofilm but the GU decreased for P4000 with both media and biofilm. These results can be explained by the fact that the post-treatment Ra in every composite decreased in P600 and P1200 with biofilm (correlating to the increase in GU) and the Ra of the BAG and BAG-F composites increased for P4000 with both media and biofilm (correlating to the decrease in GU). There was a slight but significant decrease of post-treatment Ra for the control composite in P4000 either in media or biofilm. However, the gloss of P600 and P1200 was so low, that changes at this level have little clinical relevance.

The GU decrement of the BAG composite in P2400 and P4000 was higher than that of BAG-F, but both were higher than that of the control composite after treatment in both media and biofilm. The post-treatment GU of the control composite showed little difference in media-only compared to pre-treatment GU. BAG and BAG-F filler are more susceptible to dissolution than the strontium glass filler, and thus for the composites with BAG, the higher polish leaves much of the BAG exposed on the surface, causing a roughening during dissolution. Also, the post-treatment GU of the BAG and BAG-F was less reduced in P2400 and P4000 with *S. mutans* biofilm than the media-only. This may again be due to the fact that biofilm partially covered and “protected” the BAG particles from more extensive dissolution, thus leading to less roughening compared to the surfaces aged in the media-only. Hardness is related to the resistance to indentation and is influenced by the mechanical properties of both the resin matrix and filler particles [23]. In this study, the microhardness of composites in each group showed a negative correlation with the surface roughness according to increased polishing. The microhardness of the polished surface may increase after polishing the celluloid strip-finished composite surface as it may remove the weak resin-rich surface layer [24]. A study reported that polishing procedures could cause exposure of the filler particles, which could contribute to a higher KHN. The authors, however, explained that if these procedures caused subsurface damage, the exposed weak resin matrix could compromise the hardness [25]. In the present study, the initial KHN of the BAG-F composite was lower than that of the BAG and the control composites for P600 and P1200, but the initial KHN of all composites were the same for P4000. In general, the effect of the BAG particles was minimal on the initial surface hardness, but the BAG-F composites typically had the lowest hardness when polished well, possibly due to these particles themselves being somewhat weaker than the non-F-containing BAG. However, the initial BAG-F composite surfaces gained in microhardness compared to the control

composite with increased polishing, probably because the exposed surface had an increased density of fillers after the weaker resin-rich layer was removed by the sequential polishing procedure. Though the BAG-F filler is relatively weak, it is still harder than the resin matrix, thus the overall hardness increased.

A study reported that *S. mutans* grown on the surface of resin composite increased the surface roughness and the accelerated biofilm formation in a time-dependent manner, while there were no changes in microhardness [11]. However, other studies showed that the KHN of resin composite immersed in aqueous solutions, including distilled water, decreased with immersion time [10,26]. Also, another in situ study reported that the surface KHN of resin composite both with and without biofilm decreased after two weeks [27]. The TEGDMA in the materials used in our experiment is reported to promote bacterial growth, and Bis-GMA and TEGDMA are known to enhance glycosyltransferase activity of cariogenic microorganisms [28]. Bis-GMA based polymers could be softened when soaked in organic acids, which enabled the resin matrix to promote displacement of the filler particles, resulting in a more rough surface [29,30]. Thus, the effect of bacterial biofilm on the surface of the material and the overall degradation of the resin composite could be related to a reduction in surface microhardness. In the present experiment, post-treatment KHN of BAG-F composite was lower than that of BAG although both were lower than that of the control composite. This suggested that the degradation rate of BAG-F filler may be higher than that of BAG. Also, there was only a subtle relationship between the change in KHN and the influence of *S. mutans* biofilm, which may be extrapolated to mean by that the degradation of the composite itself by the media contributed more to the reduction in surface hardness than the effect of bacterial biofilm.

The present study was based on the measurements of mechanical properties and the microscopic examination of the composite's surface before and after biofilm formation. The practical effect of *S. mutans* biofilm on the experimental composites is likely to vary depending on the viability and number of the cells in the biofilm. Disclosing the relationship between the amount of dissolved fillers and the number and viability of bacterial colonizing the composite surface may help to explain any effect of the ions dissolved from the BAG fillers on biofilm formation.

5. Conclusion

Within the limits of this in vitro study, it is concluded that both the bacterial biofilm and the media-alone can negatively affect the surface properties of experimental BAG and BAG-F composites, especially reducing hardness. The dissolution of the BAG and BAG-F fillers and the degradation of the resin matrix may increase surface roughness and decrease gloss, especially with highly polished composites. However, a somewhat lower dissolution of BAG and BAG-F fillers on the surface of composite covered with bacterial biofilm, a somewhat protective effect shown in the SEM images, may reduce the changes of surface roughness and gloss compared to aging in the media-only. It is possible that further reductions in the particle size of the BAG might mitigate some of the negative effects produced by dissolution of the particles at the surface.

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Highlights

Novel bioactive glass (BAG) and fluoride-containing BAG composites were prepared.

We investigated the effects of biofilm on the surface properties of these composites.

Both the biofilm and the media-alone can negatively affect the surface properties.

Biofilm reduced the change of surface roughness and gloss compared to the media-only.

Fig. 1.

Mean and standard deviation of Gloss Units (GU) for each group submitted to different polishing levels before and after treatment in media-only and with biofilm (n = 5).

The same superscript indicates no significant difference between before and after treatments within the same level of polishing and existence of *Streptococcus Mutans* (SM). Asterisks indicate differences between before and after treatments.

Fig. 2.

Mean and standard deviation of Knoop Hardness Number (KHN) for each group submitted to different polishing levels before and after treatment in media-only and with biofilm (n = 5).

The same superscript indicates no significant difference between before and after treatments within the same level of polishing and existence of *Streptococcus Mutans* (SM). Asterisks indicate differences between before and after treatments.

Fig. 3.

SEM images of the surface of a representative sample from the control group of resin composite. (a)–(d): P600, (e)–(h): P1200, (i)–(l): P2400, (m)–(p): P4000, (a),(e),(i),(m): composite surface after polishing, (b),(f),(j),(n): composite surface stored in the culture media free of *S. mutans* for 2 weeks. (c),(g),(k),(o): composite surface stored in the culture media with *S. mutans* biofilm for 2 weeks. (d),(h),(l),(p): biofilm formed on the composite surface after 2 weeks of aging.

Fig. 4. SEM images of the surface of a representative sample from the group of BAG-resin composite. (a)–(d): P600, (e)–(h): P1200, (i)–(l): P2400, (m)–(p): P4000, (a),(e),(i),(m): composite surface after polishing, (b),(f),(j),(n): composite surface stored in the culture media free of *S. mutans* for 2 weeks. (c),(g),(k),(o): composite surface stored in the culture media with *S. mutans* biofilm for 2 weeks. (d),(h),(l),(p): biofilm formed on the composite surface after 2 weeks of aging. The white arrows point to gaps and voids formed on the surface.

Fig. 5.

SEM images of the surface of a representative sample from the group of BAG-F-resin composite. (a)–(d): P600, (e)–(h): P1200, (i)–(l): P2400, (m)–(p): P4000, (a),(e),(i),(m): composite surface after polishing, (b),(f),(j),(n): composite surface stored in the culture media free of *S. mutans* for 2 weeks. (c),(g),(k),(o): composite surface stored in the culture media with *S. mutans* biofilm for 2 weeks. (d),(h),(l),(p): biofilm formed on the composite surface after 2 weeks of aging. The white arrows point to gaps and voids formed on the surface.

Table 1

Composition of the experimental groups

Group	Composition
Control	SG 57 wt%, OX-50 15 wt% and matrix resin 28 wt%
BAG	SG 57 wt%, BAG65 15 wt% and matrix resin 28 wt%
BAG-F	SG 57 wt%, BAG61 15 wt% and matrix resin 28 wt%

The matrix resin was made by mixing Bis-GMA and TEGDMA monomers in a 50:50 formulation with added 0.4 wt% of CQ photoinitiator, 0.8 wt% of EDMAB tertiary amine accelerator, and 0.05 wt% of BHT antioxidant.

SG : silane-treated strontium glass, SG-35SRG4000 (Bisco Inc., Schaumburg, IL, USA)

OX-50 : silane-treated aerosol-silica (Evonik Degussa, Parsippany, NJ, USA)

BAG65 : Si 65 mol%, P 4 mol% and Ca 31 mol%

BAG61 : Si 61 mol%, P 4 mol%, Ca 31 mol%, B 1 mol% and F 3 mol%

Bis-GMA : bisphenol A glycidyl methacrylate (Esstech inc., Essington, PA, USA)

TEGDMA : triethylene glycol dimethacrylate (Esstech inc., Essington, PA, USA)

CQ : camphoroquinone (Esstech inc., Essington, PA, USA)

EDMAB : 4-dimethylaminobenzoic acid ethyl ether (Acros organics, Geel, Belgium)

BHT : butylated hydroxytoluene (Sigma-Aldrich, St. Louis, MO, USA)

Table 2

Mean and standard deviation of Ra (μm) measured before and after treatments in media-only and with biofilm (n = 5)

Group:	Before Treatment			After Treatment		
	SiC	Control	BAG	BAG-F	Control	BAG
Media-only						
P600	0.472 $\pm$ 0.073 ^{Aa}	0.460 $\pm$ 0.058 ^{Aa}	0.469 $\pm$ 0.048 ^{Aa}	0.433 $\pm$ 0.059 ^{Aa*}	0.431 $\pm$ 0.088 ^{Aa*}	0.451 $\pm$ 0.046 ^{Aa}
P1200	0.264 $\pm$ 0.031 ^{Ba}	0.268 $\pm$ 0.026 ^{Ba}	0.285 $\pm$ 0.035 ^{Ba}	0.295 $\pm$ 0.033 ^{Ba*}	0.265 $\pm$ 0.026 ^{Bb}	0.313 $\pm$ 0.022 ^{Ba*}
P2400	0.090 $\pm$ 0.013 ^{Ca}	0.090 $\pm$ 0.023 ^{Ca}	0.097 $\pm$ 0.009 ^{Ca}	0.163 $\pm$ 0.022 ^{Ca*}	0.088 $\pm$ 0.019 ^{Cb}	0.165 $\pm$ 0.008 ^{Ca*}
P4000	0.049 $\pm$ 0.013 ^{Da}	0.048 $\pm$ 0.015 ^{Da}	0.048 $\pm$ 0.010 ^{Da}	0.135 $\pm$ 0.010 ^{Da*}	0.048 $\pm$ 0.013 ^{Db}	0.130 $\pm$ 0.011 ^{Da*}
Biofilm						
P600	0.466 $\pm$ 0.044 ^{Aa}	0.466 $\pm$ 0.053 ^{Aa}	0.459 $\pm$ 0.037 ^{Aa}	0.435 $\pm$ 0.049 ^{Aa*}	0.369 $\pm$ 0.052 ^{Ab*}	0.408 $\pm$ 0.057 ^{Aab*}
P1200	0.293 $\pm$ 0.049 ^{Ba}	0.284 $\pm$ 0.032 ^{Ba}	0.270 $\pm$ 0.033 ^{Ba}	0.268 $\pm$ 0.037 ^{Ba}	0.256 $\pm$ 0.053 ^{Ba*}	0.282 $\pm$ 0.031 ^{Ba*}
P2400	0.098 $\pm$ 0.014 ^{Ca}	0.089 $\pm$ 0.025 ^{Ca}	0.093 $\pm$ 0.016 ^{Ca}	0.137 $\pm$ 0.010 ^{Ca*}	0.089 $\pm$ 0.017 ^{Cb*}	0.145 $\pm$ 0.017 ^{Ca*}
P4000	0.050 $\pm$ 0.012 ^{Da}	0.042 $\pm$ 0.012 ^{Da}	0.047 $\pm$ 0.015 ^{Da}	0.105 $\pm$ 0.009 ^{Da*}	0.046 $\pm$ 0.006 ^{Db}	0.113 $\pm$ 0.015 ^{Da*}

Different letters indicate significant differences among groups.

Uppercase letters are used when comparing within columns, and lowercase letters when comparing within rows.

Asterisks indicate differences between pre- and post-treatment.

Table 3

Correlation relationships for each physical value according to increasing level of polishing from P600 to P4000 before and after treatments with and without *Streptococcus Mutans* within the same group (n = 5)[†]

Physical value:	Media-only			Biofilm		
	Control	BAG	BAG-F	Control	BAG	BAG-F
Biofilm Treatment						
Ra (µm)						
Before Tx	-0.966	-0.958	-0.968	-0.967	-0.968	-0.966
After Tx	-0.967	-0.944	-0.967	-0.962	-0.944	-0.967
GU						
Before Tx	0.966	0.965	0.914	0.952	0.945	0.909
After Tx	0.933	0.940	0.897	0.886	0.950	0.903
KHN						
Before Tx	0.425*	0.524	0.693	0.520	0.524	0.489
After Tx	0.636	0.707	0.758	0.622	0.863	0.629

[†] Spearman's rank correlation coefficient between the polishing level and each material in the same group.

* means that $P < .01$; all others were $P < .001$.