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Effect of Vital Tooth Bleaching on Surface Roughness and Streptococcal Biofilm Formation on Direct Tooth-Colored Restorative Materials

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Clinical Relevance

Vital tooth bleaching with 10% carbamide peroxide or 40% hydrogen peroxide increased both the surface roughness and biofilm formation on resin composite and resin-modified glass ionomer cement restorative materials, suggesting that existing restorations should be polished or replaced after bleaching.

SUMMARY

Objective: To compare the effect of simulated bleaching with a 10% carbamide peroxide (CP) or a 40% hydrogen peroxide (HP) system on surface roughness of resin composite and resin-modified glass ionomer cement (RMGI) and streptococcal biofilm formation on these surfaces.

Methods and Materials: Specimens of nano-filled resin composite and RMGI (n=108 each) were randomly divided into three groups (n=36 each): no treatment control, 10% CP, and 40% HP. The surface roughness values (Ra) were measured before and after treatments. The specimens in each group were randomly divided into three subgroups (n=12) and incubated with *Streptococcus mutans*, *Streptococcus sanguinis*, and trypticase soy broth control for 24 hours. Biofilm formation was quantified by crystal violet staining, and the structure was visualized by scanning electron microscopy. The differences between the mean changes in Ra between the 10% CP and 40% HP groups of each material were evaluated with an independent *t*-test. The quantity of biofilm forma-

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tion on each material was analyzed with one-way analysis of variance with the *post hoc* Tukey test (α=0.05).

Results: Surface roughness significantly increased after bleaching in all groups. There was no significant difference between the 10% CP and 40% HP groups of each material. For *S. mutans* biofilm formation, bleaching with 10% CP and 40% HP increased biofilm on both materials compared to controls. However, *S. sanguinis* biofilm formation was significantly higher on bleached resin composite but not on RMGI specimens.

Conclusions: Simulated bleaching with 10% CP or 40% HP increased both surface roughness and biofilm formation on resin composite and RMGI, except for *S. sanguinis* biofilm on RMGI.

INTRODUCTION

Tooth discoloration poses a common esthetic problem, and its treatment is in high demand. The most conservative and noninvasive procedure is vital tooth bleaching, which includes home bleaching, in-office bleaching and over-the-counter products.[1] Home bleaching using a low concentration of carbamide peroxide (CP) is the most popular procedure due to its high success rate and few side effects.[2,3] However, if the patients expect immediate results or refuse an at-home tray delivery technique, then in-office bleaching with a high concentration of hydrogen peroxide (HP) is an alternative.

Although vital tooth bleaching is a relatively safe procedure, it can have adverse effects on restorative materials.[4] A concern exists for patients with existing restorations or with carious lesions requiring restorations before bleaching treatment. Chemical softening of restorative materials caused by bleaching agents may affect clinical durability of these materials.[4] Surface texture of the restorations is also important because rough surfaces may facilitate bacterial adhesion and biofilm formation[5-7] and may also increase susceptibility to staining.[8]

Among tooth-colored restorative materials, nanofilled resin composites, other types of resin composite (such as microhybrid or nanohybrid), and resin-modified glass ionomer cement (RMGI) are the most commonly used. Previous studies reported various effects of CP or HP on surface roughness of restorative materials. While some studies showed that the surface roughness of resin composite increased after bleaching,[9,10] other studies showed insignificant changes.[11-16] Likewise, for RMGI, some studies showed that the surface roughness increased,[9,13,16] whereas others showed no effect.[12,14]

An increase in surface roughness tends to promote biofilm formation since it provides more area for bacterial adhesion and also protects bacteria from shear force and saliva flow.[17] The formation of dental biofilm begins with the adhesion of early colonizers, such as *Streptococcus sanguinis*, and is followed by the late colonizers. In an acidic environment, *Streptococcus mutans*, a major cariogenic pathogen, becomes dominant in the biofilm community and promotes the risk of dental caries initiation and progression.[18-20] Restorative materials with rough surfaces may promote biofilm formation and could potentially increase the risk for dental caries development.

The aim of this study was to evaluate the effect of two bleaching systems on the surface roughness of two direct restorative materials and the streptococcal biofilm formation on these surfaces. The direct restorative materials—a nanofilled resin composite and a RMGI—were exposed to an at-home bleaching system with 10% CP or an in-office system with 40% HP. The null hypothesis was that bleaching would not increase surface roughness of tooth-colored restorative materials and consequent biofilm formation and that different bleaching systems would not present different effects on the materials.

METHODS AND MATERIALS

Specimen Fabrication and Bleaching Procedures

Two restorative materials were used in this study: a nanofilled resin composite material (Filtek Z350, 3M ESPE, St Paul, MN, USA) and an RMGI (Fuji II LC, GC Corp, Tokyo, Japan). One hundred and eight specimens of each material in shade A2 were fabricated into disks of 5 mm in diameter and 2 mm thick. The materials were inserted into metal molds positioned on a transparent plastic matrix strip and a glass slab. A second transparent plastic matrix strip and glass slab were used to compress the restorative materials. Each specimen was cured with an LED light (Demi, SDS Kerr, Danbury, CT, USA) at 800 mW/cm^2 for 40 seconds. All specimens were polished in a stepwise manner with medium, fine, and superfine polishing discs (Sof-Lex, 3M ESPE) on a slow-speed hand piece rotating in one direction and cleaned in distilled water in an ultrasonic cleanser for five minutes. All specimens were stored in artificial saliva at 37°C for 24 hours.

Table 1: *Composition and Application of Materials Used in This Study*

	Composition	Application
Nanofilled resin composite (Filtex Z350, 3M ESPE Dental Products, St Paul, MN, USA)	Matrix: Bis-GMA, UDMA, TEGDMA and Bis-EMA	Curing time: 20 s
	Filler: combination of aggregated Zr/Si cluster filler (0.6-1.4 μm) and nonaggregated 20-nm Si filler (filler volume: 63.3%)	
Resin-modified glass ionomer cement (Fuji II LC, GC Corp, Tokyo, Japan)	Powder: fluoroaminosilicate glass	Mixing time: 10 s
	Liquid: polyacrylic acid, tartaric acid, distilled water, camphoquinone, dibutyl hydroxy toluene, and three-resin complex (mainly HEMA)	Working time: 3 min, 15 s
		P/L ratio 0.33 g/0.10 g
		Curing time: 20 s
At-home bleaching (Opalescence PF, Ultradent Products, South Jordan, UT, USA)	10% carbamide peroxide,	8 h/session, 14 sessions
	potassium nitrate, 0.11% fluoride ion,	
	carbopol, glycerine, flavoring (pH=6.7)	
In-office bleaching (Opalescence Boost, Ultradent Products)	40% hydrogen peroxide,	Two 20-min applications for a total of 40 min of treatment time
	potassium nitrate, 0.11% fluoride ion, carbopol, glycerine, flavoring (pH=7)	

Abbreviations: Bis-GMA, bis-phenol A-glycidyl dimethacrylate; UDMA, urethane dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; Bis-EMA, bis-phenol A-ethoxylated dimethacrylate; Zr, Zirconium; Si, Silicon; HEMA, hydroxyethylmethacrylate; min, minute(s); s, second(s); g, gram(s); P/L ratio, powder to liquid ratio.

The specimens of each restorative material type were randomly divided into three groups (n=36 each): no treatment control, 10% CP, and 40% HP treatment groups. The control specimens were stored in artificial saliva for 112 hours at 37°C, and the artificial saliva was changed daily. In the 10% CP group, specimens were bleached with 10% CP (Opalescence PF, Ultradent Products Inc, South Jordan, UT, USA) for 14 cycles of eight-hour applications to simulate home bleaching conditions according to manufacturer recommendations. Between each cycle, bleaching agents were rinsed off with distilled water for 20 seconds. In the 40% HP group, specimens were bleached with 40% HP (Opalescence Boost, Ultradent Products) for two cycles of 20-minute applications to simulate in-office bleaching according to manufacturer recommendations. After bleaching, all specimens were rinsed off with distilled water for 20 seconds and air-dried for 30 seconds. Information on material compositions and bleaching material applications is shown in Table 1.

Surface Roughness Measurement

The surface roughness values (Ra) were measured by a high-resolution three-dimensional optical surface measurement device (InfiniteFocusSL, Alicona Imaging GmbH, Graz, Austria) at 50× magnification in five different areas before and after treatment procedures. Specimens were fixed with a special jig to place them in the same positions for the measurements. The average surface roughness (Ra) was determined and recorded.

Bacterial Cultures

Bacterial cultures were prepared from frozen stocks of *S. mutans* ATCC25175 and *S. sanguinis* ATCC10556 as described by Ittatirut and others.[21] *S. mutans* and *S. sanguinis* were cultured in trypticase soy agar plates and incubated at 37°C with 5% CO_2 for 48 hours. For each experiment, a single colony was inoculated into sterile trypticase soy broth and incubated at 37°C with 5% CO_2 for 16 hours. The OD_{550nm} of the cultures was adjusted to 0.1 as measured by a spectrophotometer (Pharmacia LKB Biotechnology Inc, Uppsala, Sweden) and incubated at 37°C for two hours until the OD_{550nm} reached 0.3. The cultures were then centrifuged and resuspended in trypticase soy broth with 4% sucrose for biofilm formation assays.

Biofilm Formation Assays

The specimens were mounted into 96-well plates and disinfected with ethylene oxide gas. Each group of the specimens was randomly divided into three subgroups (n=12) for biofilm formation assays with *S. mutans*, *S. sanguinis*, and no bacteria control. The assay was done as described by Ittatirut and others[21] with minor modifications. Each well containing a specimen was filled with 100 μL of filter-sterilized artificial saliva and incubated at 37°C for two hours. Then 100 μL of bacterial suspensions with 4% sucrose in trypticase soy broth were dispensed onto each specimen, except for the control group, which received only media without bacteria. All specimens were incubated at 37°C with 5% CO_2 for 24 hours.

Table 2: *Surface Roughness Values (nm) Before and After Bleaching*

Restorative Materials	Bleaching Agents	Before Bleaching		After Bleaching		*p*-Value[a]
		Mean	SD	Mean	SD	
Resin composite	10% CP	183.65	12.48	189.21	12.35	<0.001
Resin composite	40% HP	179.41	15.56	185.13	15.52	<0.001
RMGI	10% CP	375.75	59.67	443.98	65.54	<0.001
RMGI	40% HP	365.16	63.28	427.52	75.70	<0.001

Abbreviations: CP, carbamide peroxide; HP, hydrogen peroxide; RMGI, resin-modified glass ionomer cement.
[a] *Paired* t*-test.*

The total amount of biofilm formation (n=9 for each subgroup) was quantified by the crystal violet assay. The amount of biofilm was measured by the optical density of extracted crystal violet in the destaining solution at 595 nm (OD_{595nm}) with a microplate reader (Biochrom Anthos Zenyth 200rt, Biochrom US, Holliston, MA, USA). Each optical density value of the biofilm group was subtracted by the mean optical density of the respective controls without bacteria to remove background value.

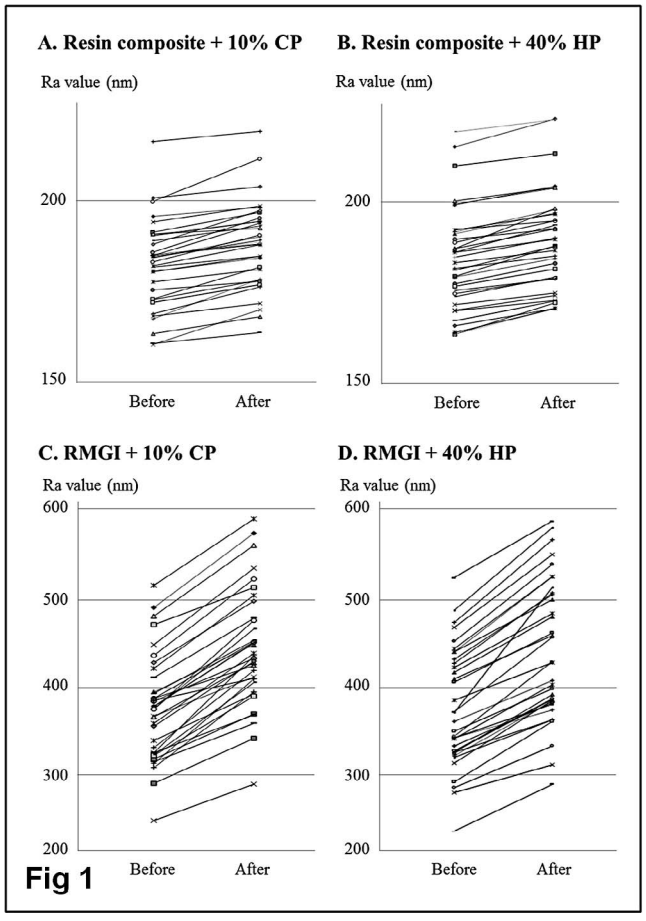

Figure 1. *Bleaching significantly increased surface roughness of both resin composite and resin-modified glass ionomer cement (RMGI). Scatter plots of paired data (before and after bleaching) of surface roughness values are shown. (A): Resin composite before and after bleaching with 10% carbamide peroxide. (B): Resin composite before and after bleaching with 40% hydrogen peroxide. (C): RMGI before and after bleaching with 10% carbamide peroxide. (D): RMGI before and after bleaching with 40% hydrogen peroxide.*

Three specimens of each group were prepared and examined with a scanning electron microscope at 6000× magnification (model JSM-5410LV, JEOL Ltd, Tokyo, Japan).

Statistical Analysis

Statistical calculations were performed with SPSS version 17.0 software (SPSS Inc, Chicago, IL, USA). The differences in surface roughness values between before and after treatments were evaluated with a paired *t*-test. The differences of mean changes in surface roughness values between the 10% CP and 40% HP groups of each material were evaluated with an independent *t*-test. The amount of biofilm formation among groups of the same material and bacterial species was evaluated with one-way analysis of variance with the *post hoc* Tukey test. The correlation between surface roughness and biofilm formation was analyzed by linear regression. Statistical significance level was set at $\alpha = 0.05$.

RESULTS

Surface Roughness—When comparing the surface roughness values before and after bleaching within each group, we found that surface roughness significantly increased after bleaching treatments for all groups ($p<0.001$) (Table 2; Figure 1). No significant difference was observed when the mean changes in surface roughness were compared between 10% CP and 40% HP for each material (Table 3).

Biofilm Formation—On resin composite specimens, bleaching with 10% CP or 40% HP increased both *S. mutans* and *S. sanguinis* biofilm formation when compared to unbleached specimens ($p<0.001$), with no significant difference between the 10% CP and 40% HP groups ($p=0.661$). *S. mutans* biofilm

Table 3: *Mean Changes in Surface Roughness Values (nm) Between 10% Carbamide Peroxide and 40% Hydrogen Peroxide*

Restorative Materials	10% Carbamide Peroxide		40% Hydrogen Peroxide		*p*-Value[a]
	Mean	SD	Mean	SD	
Resin composite	5.56	2.75	5.72	2.11	0.931
RMGI	67.98	19.95	62.11	21.49	0.096

Abbreviation: RMGI, resin-modified glass ionomer cement.
[a] *Independent* t-*test.*

formation on bleached RMGI specimens were also increased ($p<0.001$) with no significant difference between 10% CP and 40% HP ($p=0.487$). However, no significant difference was observed for *S. sanguinis* biofilm formation between bleached and unbleached RMGI specimens ($p=0.063$) (Table 4). When we analyzed the relationship between surface roughness and biofilm formation, regardless of bleaching treatments, simple regression showed a significant correlation only in the RMGI and *S. mutans* group ($p=0.013$, $r=0.473$) (Figure 2).

Representative scanning electron micrographs of biofilm structure are shown in Figure 3 (resin composite) and Figure 4 (RMGI). As expected, the control groups without bacteria did not show any biofilm formation (Figure 3A,B,C and Figure 4A,B,C). For resin composite specimens, *S. mutans and S. sanguinis* biofilm formation was observed in all groups, but the number of cells in unbleached groups appeared lower than the bleached groups (Figure 3D,E,F,G,H,I) For RMGI specimens, all groups showed similar *S. mutans and S. sanguinis* biofilm formation (Figure 4D,E,F,G,H,I).

DISCUSSION

In this study, we found that surface roughness of both materials significantly increased after bleaching treatment for all groups with no significant difference between 10% CP and 40% HP groups. For *S. mutans* biofilm formation, bleaching with 10% CP and 40% HP increased biofilm on both materials compared to the control group. However, for *S. sanguinis* biofilm, there was significantly higher biofilm formation on bleached resin composite but not on RMGI specimens. Hence, the findings of this study reject the first part of the null hypothesis, showing that the bleaching systems used promoted increased surface roughness and biofilm formation on both materials tested. The second part of the null hypothesis, however, is accepted since no difference in effect between the two bleaching systems used was observed. To our knowledge, this is the first study that evaluates the effect of bleaching on biofilm formation of both *S. mutans* and *S. sanguinis* on tooth-colored restorative materials.

Vital bleaching is a popular treatment option for discolored teeth due to its high success rate, ease of

Table 4: *Streptococcal biofilm formation on restorative materials bleached with 10% carbamide peroxide and with 40% hydrogen peroxide.*

Restorative materials	Bacteria	Amount of biofilm ($OD_{595\ nm}$)			ANOVA	Tukey test		
			Mean	SD	p-value	pairwise comparison		p-value
Resin composite	*S. mutans*	Unbleached	0.178	0.086	<0.001	Unbleached	10% CP	<0.001
		10% CP	0.442	0.134		Unbleached	40% HP	<0.001
		40% HP	0.493	0.119		10% CP	40% HP	0.661
Resin composite	*S. sanguinis*	Unbleached	0.173	0.063	<0.001	Unbleached	10% CP	<0.001
		10% CP	0.774	0.129		Unbleached	40% HP	<0.001
		40% HP	0.340	0.123		10% CP	40% HP	0.487
RMGI	*S. mutans*	Unbleached	0.656	0.080	<0.001	Unbleached	10% CP	<0.001
		10% CP	0.985	0.115		Unbleached	40% HP	<0.001
		40% HP	0.973	0.166		10% CP	40% HP	0.977
RMGI	*S. sanguinis*	Unbleached	0.746	0.149	0.063	Unbleached	10% CP	0.054
		10% CP	0.922	0.183		Unbleached	40% HP	0.273
		40% HP	0.859	0.117		10% CP	40% HP	0.658

Abbreviations: CP, carbamide peroxide; HP, hydrogen peroxide; RMGI, resin-modified glass ionomer cement.

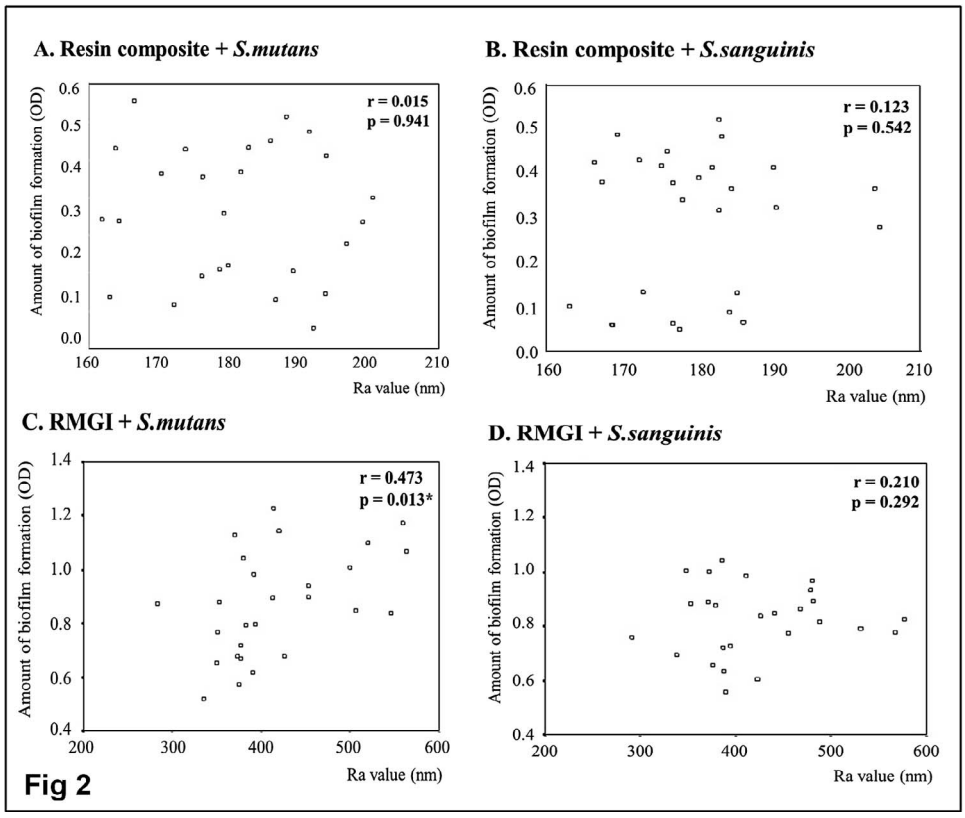

Figure 2. *Relationship between surface roughness values (Ra) and amount of biofilm formation (optical density from crystal violet staining assays) were analyzed with linear regression analysis. (A): Resin composite and* S. mutans *biofilm. (B): Resin composite and* S. sanguinis *biofilm. (C): Resin-modified glass ionomer cement (RMGI) and* S. mutans *biofilm. (D): RMGI and* S. sanguinis *biofilm.* r*, correlation coefficient;* *p $<$ *0.05 is considered significant.*

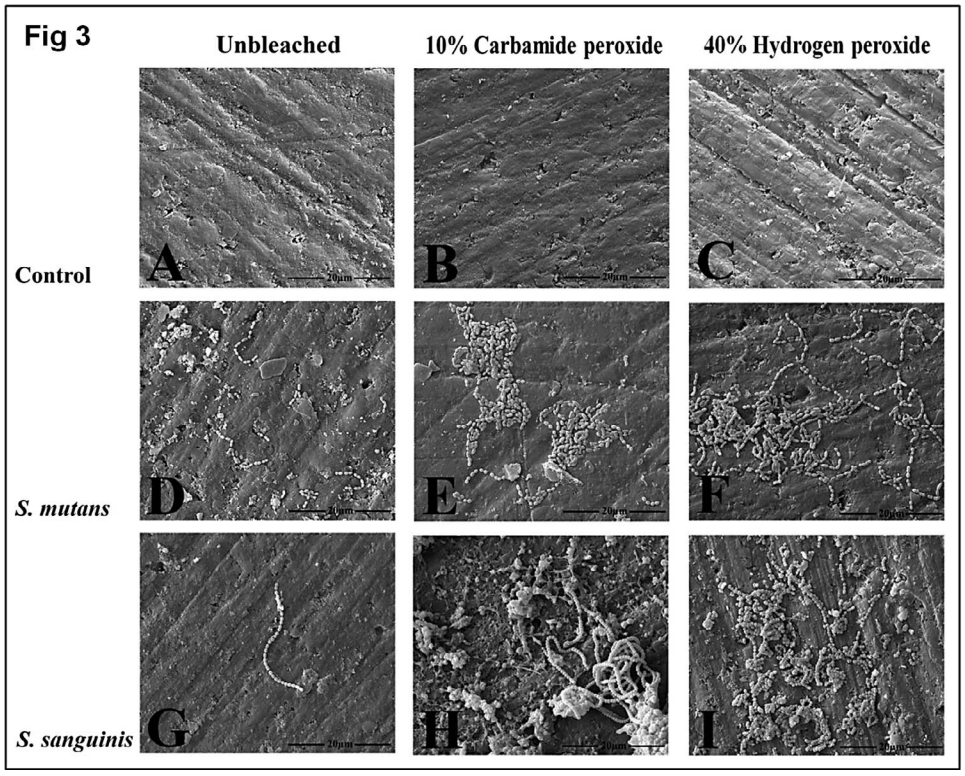

Figure 3. *Scanning electron microscopic images of representative examples of resin composite specimens show biofilm structure on the surfaces. (A): Unbleached without bacteria. (B): Bleached with 10% carbamide peroxide without bacteria. (C): Bleached with 40% hydrogen peroxide without bacteria. (D): Unbleached with* S. mutans*. (E): Bleached with 10% carbamide peroxide with* S. mutans*. (F): Bleached with 40% hydrogen peroxide with* S. mutans*. (G): Unbleached with* S. sanguinis*. (H): Bleached with 10% carbamide peroxide with* S. sanguinis*. (I): Bleached with 40% hydrogen peroxide with* S. sanguinis.

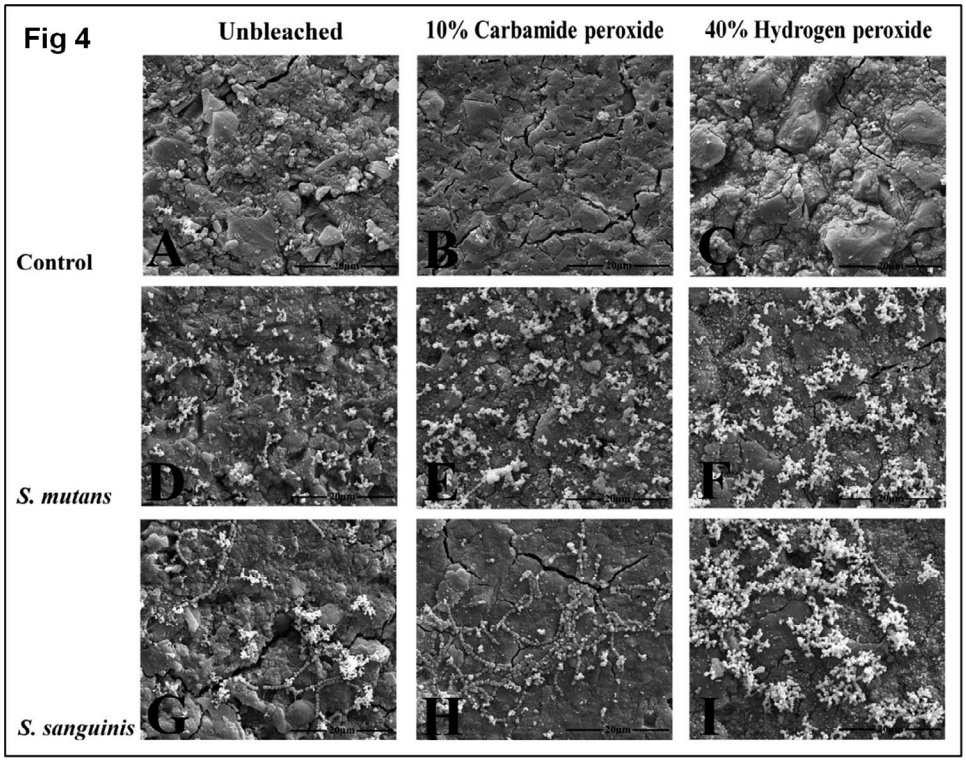

Figure 4. *Scanning electron microscopic images of representative examples of resin-modified glass ionomer cement specimens show biofilm structure on the surfaces. (A): Unbleached without bacteria. (B): Bleached with 10% carbamide peroxide without bacteria. (C): Bleached with 40% hydrogen peroxide without bacteria. (D): Unbleached with* S. mutans. *(E): Bleached with 10% carbamide peroxide with* S. mutans. *(F): Bleached with 40% hydrogen peroxide with* S. mutans. *(G): Unbleached with* S. sanguinis. *(H): Bleached with 10% carbamide peroxide with* S. sanguinis. *(I): Bleached with 40% hydrogen peroxide with* S. sanguinis.

use, and conservativeness. However, bleaching could have adverse effects on existing restorations. Surface roughness is an important property that may affect clinical longevity of restorations, susceptibility to staining, bacterial adhesion, and biofilm formation.[5-7] Previous studies showed an increase in surface roughness of direct tooth-colored restorations after bleaching treatments.[9,10] Moreover, Mor and others[22] showed that bleaching agents may affect adherence of cariogenic microorganisms to the outer surfaces of resin composite restorations.

Our study showed that treatment with 10% CP or 40% HP increased surface roughness of nanofilled resin composite. Compared to previous studies on resin composite, this is in agreement with certain studies[9,10] but differs from others.[11-16] The different results may be due to the use of different types of resin composite, bleaching agents, bleaching systems, and methods of surface roughness measurement. Among the three reports that studied nanofilled resin composite, our results are similar to those using similar measurement methods reported by Markovic and others,[9] who used a three-dimensional optical surface measurement device to examine the effect of 16% CP, 22% CP, and 38% HP, and by Rattacaso and others,[10] who used a contact profilometer to evaluate the effect of 16% CP. In contrast, our results differ from the report of Yu and others,[16] who used scanning electron microscopy to evaluate the effect of 15% CP.

Similarly, we observed that the surface roughness of RMGI also increased after 10% CP or 40% HP application. This result is similar to the findings of Turker and Biskin[13] but is different from those of Wattanapayungkul and Yap.[14] Besides differences in roughness measurement methods among these studies, different bleaching systems differ not only in concentrations of bleaching agents but also in pH and application procedures. It is reasonable to conclude that the effect of bleaching on material surfaces may also be system dependent.

The mechanisms by which bleaching agents could adversely affect resin composite and RMGI are not well understood. Peroxides could induce oxidative cleavage of polymer chains, especially the unreacted double bonds that are the most vulnerable parts of the polymers.[23-25] Durmer and others[26] found that HP reacts with not only the unreacted C-C double bonds but also the C-C single bond in a polymer network in resin composite. Free radicals induced by peroxides may also have an impact on the resin–filler interface and cause a filler–matrix debonding.[23,27,28] Furthermore, water uptake may result in stress corrosion and debonding of fillers.[29]

The effect of bleaching on the surfaces of restorative materials could influence bacterial biofilm

formation. In this study, we used *S. sanguinis and S. mutans* as the representative species of early colonizers and major cariogenic pathogens, respectively.[18-20] On resin composite specimens, bleaching with 10% CP or 40% HP increased both *S. sanguinis* and *S. mutans* biofilm formation compared with unbleached specimens. In contrast, bleaching increased only *S. mutans* biofilm formation on RMGI specimens. Because surface roughness may play an important role in biofilm formation of oral bacteria, we evaluated the relationship between surface roughness and biofilm formation. We found a correlation between *S. mutans* biofilm formation and surface roughness of RMGI. This correlation was similarly observed in an earlier study on enamel surfaces from our group[21] and a study on restorative materials from Filiz and others.[6] Unlike *S. mutans*, we did not observe a correlation between *S. sanguinis* biofilm and surface roughness both in this study and in our previous study on enamel surfaces.[21] This may partly explain why we found an increase in *S. mutans* but not *S. sanguinis* biofilm formation on RMGI with greater surface roughness after bleaching. Moreover, other factors, such as pH and changes in surface chemistry after bleaching, could also influence biofilm formation.[21]

Since cariogenic bacteria are an essential causative factor for the pathogenesis of dental caries, an increase in bacterial adhesion and biofilm formation on restorative materials could potentially increase the risk for secondary caries. Previous studies suggest that reduction of oral mutans streptococcal colonization is associated with lower caries increment[30] and that the use of restorative materials or adhesives with antimicrobial properties may lower the risk of secondary caries.[31,32] However, direct evidence for the association between bacterial adhesion or biofilm formation on restorative materials and secondary caries is still lacking.[33] As secondary caries is a common cause of restoration failures, further investigations into this issue are needed, especially studies in clinical settings.[33,34]

Within the limitation of our *in vitro* study of single-species bacterial biofilm, our results imply that both high and low concentrations of peroxide bleaching agents in in-office and home bleaching systems, respectively, could similarly increase surface roughness and biofilm formation on tooth-colored restorations. Thus, dentists should pay attention to planning appropriate sequences of treatments and should polish existing tooth-colored restorations after bleaching. Nevertheless, additional research on multispecies biofilm and clinical studies is required.

CONCLUSIONS

Within the limitations of this study, it is possible to conclude that the bleaching systems used, 10% CP or 40% HP, significantly increased both the surface roughness and the streptococcal biofilm formation on resin composite and resin-modified glass ionomer cement.

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Conflict of Interest

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

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