Dental Research Journal

Original Article

Effect of bleaching protocols on surface roughness and biofilm formation on silorane-based composite resin

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Received: June 2017
Accepted: October 2018

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ABSTRACT

Background: Knowledge about the effect of bleaching on behavior of composite resins is important to find a suitable composite resin for restoration of teeth undergoing bleaching. This study aimed to assess the effect of different bleaching protocols on surface roughness and biofilm formation on a silorane-based composite resin.

Materials and Methods: In this *in vitro* experimental study, 60 silorane-based composite resin samples measuring 3 mm in thickness and 6 mm in diameter were fabricated and polished. They were then randomly divided into four groups (n = 15). In Group 1, samples were stored in distilled water as control. Samples in Groups 2, 3, and 4 were subjected to bleaching with 15% carbamide peroxide, 35% hydrogen peroxide, and 35% hydrogen peroxide activated by light, respectively. Surface roughness was measured using a profilometer. *Streptococcus mutans* cultured in brain-heart infusion broth was used for the assessment of biofilm formation on the samples. The bacterial colonies were counted using the pure-plate technique. Data were analyzed using one-way ANOVA and *post hoc* Tukey's tests. Regression model was used to assess the association between surface roughness and biofilm formation ($P < 0.05$).

Results: The mean surface roughness of the four groups was not significantly different ($P = 0.11$); however, a significant difference was noted in the mean biofilm formation among the groups ($P = 0.00$).

Conclusion: Bleaching decreased biofilm formation. The lowest biofilm formation was noted in the group subjected to light-activated 35% hydrogen peroxide. Increased surface roughness enhanced biofilm formation to a certain level; excessive roughness did not increase biofilm formation.

Key Words: Biofilm, composite resin, silorane, tooth bleaching

INTRODUCTION

Vital and nonvital tooth bleaching has a long and successful history. Bleaching treatment offers in the forms of at-home or in-office bleaching with the use of carbamide peroxide (CP) and hydrogen peroxide (HP), respectively. About 15% CP is the most commonly used bleaching agent for at-home bleaching, while HP is the most effective bleaching agent for elimination of internal stains in the office setting.[1]

It has been demonstrated that tooth bleaching is relatively safe in terms of potential changes in tooth

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How to cite this article: Bahari M, Ebrahimi Chaharom ME, Daneshpooy M, Gholizadeh S, Pashayi H. Effect of bleaching protocols on surface roughness and biofilm formation on silorane-based composite resin. Dent Res J 2019;16:264-70.

structure. However, some concerns still exist regarding the adverse effects of bleaching agents on restorative materials and their adhesion to dental tissues.[2-4]

Changes in surface roughness and microhardness are commonly used to study the possible adverse effects of bleaching agents on restorative materials. Increase in surface roughness enhances the accumulation of food and biofilm formation and increases the risk of periodontal disease. Evidence shows that bacterial accumulation directly depends on surface roughness.[5,6] However, studies on the effect of bleaching agents on the surface roughness of dental materials have reported controversial results.[7-9] The effects of bleaching on resin-based materials depend on the type of resin, composition of the bleaching gel, and duration and frequency of exposure.[8,10,11]

To overcome the most important shortcoming of conventional composite resins, which is their polymerization shrinkage, silorane-based composite resins were introduced to the market, which has ring-opening polymerization mechanism. It has been shown that silorane-based composite resins have less bacterial adhesion than methacrylate-based ones due to greater hydrophobicity.[12]

Knowledge about the effect of bleaching on the properties and behavior of composite resins is important to use the most suitable composite resin for restoration of teeth undergoing bleaching. By doing so, the need for composite resin restoration exchange due to possible complications caused by bleaching treatment is obviated. This study sought to assess the effect of different tooth bleaching protocols using 15% CP, 35% HP, and 35% HP activated by light on the surface roughness and biofilm formation on a silorane-based composite resin.

MATERIALS AND METHODS

In this *in vitro*, experimental study, A3 shade of Filtek P90 (3M ESPE, St. Paul, MN, USA) was used to fabricate 60 cylindrical samples measuring 3 mm in height and 6 mm in diameter. To determine the sample size, a pilot study was performed considering the power of 80% and $\alpha = 0.05$. Hence, 15 samples were determined for each group and 60 for the study.

A cylindrical acrylic mold was used for this purpose. The mold was filled with composite resin, and its surface was covered with a polyester strip and a glass slab. It was then compressed with 500 g load for 30 s for the excess material to leak out and obtain parallel surfaces. Load was then discontinued and the glass slab was removed. The samples were then light cured for 20 s using Demetron A2 light-curing unit (Kerr, WI, USA) with a light intensity of 1000 mW/cm^2 according to the manufacturer's instructions. Then, the samples were removed from the mold and immersed in distilled water at 37°C for 24 h. Samples were then polished with coarse, medium, fine, and extrafine aluminum oxide Sof-Lex discs (3M ESPE, St. Paul, MN, USA), respectively. After polishing, the samples were immersed in the deionizing solution to ultrasonically remove the residues and were then randomly divided into four groups ($n = 15$) as follows:

- Group 1: The samples were stored in distilled water at 37°C for 2 weeks as the control
- Group 2: The samples were subjected to bleaching with 15% CP (Everbrite at-home tooth whitening kit, Dentamerica, City of Industry, CA, USA) for 2 h a day for a total of 2 weeks
- Group 3: The samples were subjected to bleaching with 35% HP (Whiter Image, Pac-Dent International Inc., CA, USA) as recommended by the manufacturer every 3–5 days for 30 min for a total of 2 weeks
- Group 4: The samples were subjected to bleaching with 35% HP (Everbrite in-office tooth whitening kit, Dentamerica, City of Industry, CA, USA) activated by light using LITEX 686 LED Curing and Whitening System (Dentamerica, City of Industry, CA, USA) for 40 min according to the manufacturer's instructions.

Samples were cleaned with a soft toothbrush and distilled water for 1 min to eliminate the bleaching agents from the tooth surfaces. This was done daily after bleaching in Group 2, after each cycle of bleaching in Group 3, and after the completion of bleaching in Group 4.

Measurement of surface roughness

Surface roughness of the samples was measured using a profilometer (Talysurf CLI 1000, Leicester, England). The device was calibrated as recommended by the manufacturer. Each sample was subjected to measurements in triplicate, and the mean value was calculated and reported. For the purpose of standardization, surface roughness was measured at the center of samples and at two other points with 2-mm distance from the center.

Assessment of biofilm formation

The pour-plate technique was used to count *Streptococcus mutans* colonies cultured in brain–heart infusion broth. The bacteria were incubated at 37°C for 24 h. After centrifugation, the supernatant was discarded and the bacteria were washed with saline and dried. Staining was done with 2% crystal violet for 5 min and optical density was read at 620 nm. For counting the bacteria after incubation, each composite resin sample was gently rinsed with phosphate-buffered saline solution and placed in a sterile 1.5-mL microplate. The tubes were vortexed for 90 min at 2500 rpm to separate the biofilm matrix. The number of colonies was counted and reported in colony-forming units (CFU). Conflicting factors for the formation of biofilms were inadequate and noncalibrated washing, which eliminated by controlling the washing and calibration of the device by biochemical methods. To assess the association of surface roughness and biofilm formation, the samples were categorized into three groups in terms of surface roughness (≤0.5, 0.6–0.9, ≥1 μm).

Statistical analysis

The surface roughness and biofilm formation data were statistically analyzed using the one-way ANOVA and Tukey's tests through SPSS version 16 (SPSS Inc. Chicago, IL, USA). The regression model was used to assess the correlation between surface roughness and biofilm formation. The assessment of the correlation between surface roughness and biofilm formation following the classification of samples into three groups of ≤0.5, 0.6–0.9, ≥1 μm in terms of surface roughness was done using one-way ANOVA and Tukey's *post hoc* tests. $P < 0.05$ was considered statistically significant.

RESULTS

Table 1 summarizes the mean ± standard deviation of surface roughness (μm) and biofilm formation (CFUs/mL) after different bleaching protocols.

Table 1: Mean±standard deviation of surface roughness (μm) and biofilm formation colony-forming units/mL after different bleaching protocols

Group	Mean±SD	
	Surface roughness(μm)	Biofilm formation (CFU/mL)
Control	6636^{A}±0.11201	9086.3×10^{3a}±710.54×10^{3}
LAHP	6500^{A}±0.32522	6151.3×10^{3b}±476.42×10^{3}
35% HP	9462^{A}±0.57244	7323.3×10^{3c}±232.04×10^{3}
15% CP	6571^{A}±0.25635	8369.7×10^{3d}±281.49×10^{3}

Different superscripts mean statistically significant differences ($P<0.05$).
HP: Hydrogen peroxide; CP: Carbamide peroxide; SD: Standard deviation; CFU: Colony-forming units; LAHP: Light-activated HP

The Kolmogorov–Smirnov test confirmed normal distribution of the data ($P > 0.05$). The one-way ANOVA found no significant difference in the mean surface roughness of the four groups ($P = 0.11$). However, the four groups were significantly different in terms of biofilm formation ($P = 0.00$).

Pair-wise comparisons of biofilm formation among the groups using Tukey's test revealed that:

- The amount of biofilm in the light-activated 35% HP and 35% HP groups ($P = 0.00$) and in 15% CP group ($P = 0.001$) was significantly lower than that in the control group
- The amount of biofilm in the light-activated 35% HP group was significantly lower than that in 35% HP ($P = 0.00$) and 15% CP ($P = 0.00$) groups
- The amount of biofilm in 35% HP group was significantly lower than that in 15% CP group ($P = 0.00$) [Figure 1].

The Pearson's correlation test found no significant association between surface roughness and biofilm formation ($P > 0.05$). However, assessment of the correlation between surface roughness and biofilm formation using the one-way ANOVA following the classification based on surface roughness of ≤0.5, 0.6–0.9, and ≥1 μm revealed significant differences in biofilm formation ($P = 0.03$).

Pairwise comparisons using Tukey's test showed that the amount of biofilm in samples with 0.6–0.9 μm surface roughness was significantly higher than that in samples with <0.5 μm and >1 μm surface roughness ($P = 0.04$). Furthermore, the amount of biofilm was equal in samples with <0.5 μm and >1 μm surface roughness [Figure 2].

DISCUSSION

Surface roughness is an important clinical property with a confirmed effect on dental esthetics and health. High surface roughness may lead to composite resin wear, discoloration, or bacterial accumulation. Several studies have assessed the effect of bleaching agents on the surface roughness of composite resins. Some authors have reported no change in surface roughness of restorative materials after bleaching.[9,13-16] Some others, however, have reported a reduction[17] and some have shown an increase[18,19] in surface roughness as the result of bleaching treatment. Considering the different composition and structure of silorane-based composite resins compared to conventional types, the current study assessed the effect of different bleaching

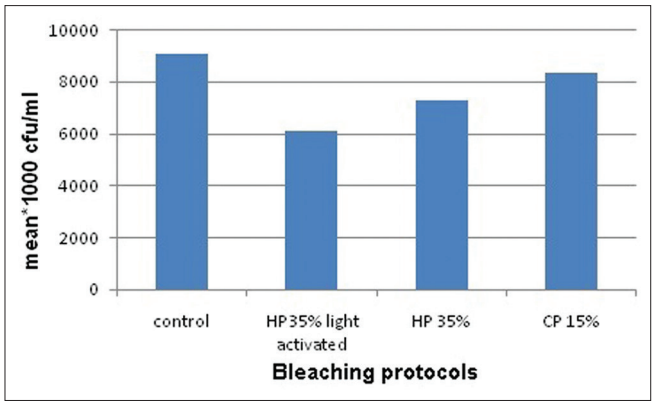

Figure 1: Comparison of surface roughness of silorane-based composite resin samples subjected to different bleaching protocols.

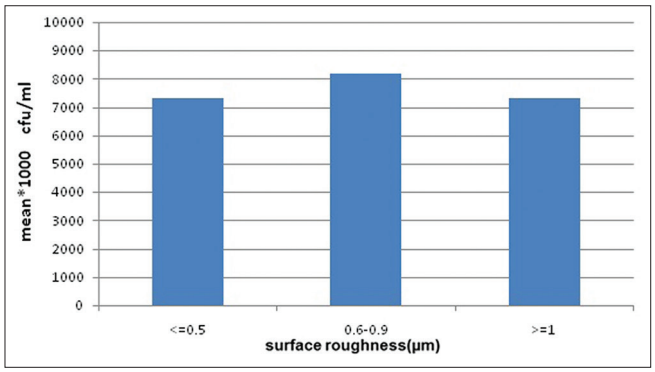

Figure 2: Comparison of biofilm formation based on surface roughness of silorane-based composite resin samples (correlation of surface roughness and biofilm formation).

protocols on the surface roughness and biofilm formation on a silorane-based composite.

Regarding surface roughness, no significant difference was found in the mean surface roughness values of the four groups. Similarly, Schemehorn *et al*. reported that 6% HP had no significant effect on the surface morphology of composite resins.[20] Wattanapayungkul *et al*. only found insignificant differences in surface roughness between the control and bleached groups.[21] However, some studies showed that 10% and 16% CP caused a small but significant increase in surface roughness and porosity of microfilled and hybrid composite resins.[15]

Free radicals produced by the peroxides may affect the resin–filler interface and cause filler–matrix detachment.[21] In other words, free radicals eventually form water and accelerate the hydrolytic degradation of composite resins. The latter can also cause bond failure between the resin matrix and filler particles and lead to separation and debonding of filler particles, which further increase the surface roughness of composite resin.[22] However, as mentioned earlier, silorane-based composite resins are more hydrophobic and have lower water sorption due to their cationic rings. The size of the filler particles is one of the determinative factors for surface roughness and polishability of restorative materials.[23] Since the same composite resin was used in all groups in the current study, there were no significant differences in surface roughness.

Another important finding of the present study was that the amount of biofilm in the control group was greater than that in other groups. On the other hand, bleaching decreased biofilm formation on Filtek P90 silorane-based composite resin. Hydrophobicity is among the factors responsible for bacterial adhesion. Bacteria with hydrophobic surfaces such as *S. mutans* have high affinity for hydrophobic materials. Since the same composite resin was used in all groups in the current study, hydrophobicity cannot explain the differences among the groups; however, the bleaching protocols might have decreased the hydrophobicity of the samples. Thus, one major cause of reduction in biofilm formation in the bleached groups may be the antibacterial effects of the bleaching agents. Some previous studies have shown that HP-based bleaching agents have bactericidal properties.[24-26]

Many factors influence biofilm formation, including surface roughness, surface free energy, and the chemical combination of the surface. It has been shown that increasing the surface free energy increases the bacterial adhesion.[27] Upon bleaching of the methacrylate-based composite resins, their surface free energy increases and this can enhance biofilm formation. It seems that the effect of bleaching agents on the surface free energy of silorane-based composite resins is different from that of methacrylate-based composite resins due to the completely different chemical composition and higher hydrophobicity of the former group. However, further studies are required to confirm this hypothesis.

Another important finding of this study was that biofilm formation in light-activated 35% HP group was significantly lower than that in 35% HP and 15% CP groups. Furthermore, the amount of biofilm formation in 35% HP group was less than that in 15% CP group. Fillers as well as resin matrix of composite resins may affect biofilm formation.[28] Ono *et al*. showed that most bacteria were eliminated

when exposed to a suspension of composite resin monomer.[28] Tabatabaee *et al*. demonstrated that bleaching agents enhanced the release of monomers from composite resins.[29] Polydorou *et al*. assessed the effect of 35% HP and 15% CP on different composite resins and noticed that the release of monomers following exposure to HP was greater compared to CP.[7] A possible explanation may be that higher amounts of monomers released from the samples bleached with HP resulted in a greater reduction in biofilm formation compared to CP group. In addition, Yuan *et al*. reported that cold-light bleaching treatment inhibited biofilm formation and decreased viable bacteria count.[24]

Furthermore, the current study assessed the correlation between surface roughness and biofilm formation and showed that samples with 0.6–0.9 μm surface roughness had greater biofilm formation than those with other surface roughness values. There is some controversy regarding the effect of bleaching on bacterial adhesion. Some studies have shown a rise in bacterial adhesion with an increase in surface roughness.[17,19] Some others have shown no relationship between surface roughness and bacterial adhesion.[6,30] The acceptable threshold of surface roughness is believed to be 0.2 μ and if restorative materials have surface roughness higher than the threshold, there will be an increased risk of plaque accumulation, gingival inflammation, and dental caries.[23,31] The results of the present study showed that increased surface roughness enhanced biofilm formation to a certain level. However, Ikeda *et al*. demonstrated that smooth surfaces of composite resins with low surface roughness caused lower bacterial adhesion than rougher surfaces.[32]

Mei declared that *S. mutans* bacteria attach to rough surfaces less tightly.[33] Therefore, contradictory reports exist regarding the actual effect of surface roughness on bacterial adhesion and biofilm formation. An *in vitro* study showed that surface roughness above several hundreds of nanometers generally increases the adhesion of *Streptococcus sanguinis* and *S. mutans* to the surfaces of composite resins (Ra 150–560 nm).[34] According to some studies, in surface roughness values <200 nm, surface roughness has no significant effect on plaque aggregation and microbial accumulation on titanium abutments *in vivo*.[33,35] It has no significant effect on attachment and colonization of *Staphylococcus epidermidis* on silicone surfaces *in vitro*, either.[36] Therefore, probably, a specific roughness value or range exists below which the adhesion forces are not strong enough to create sufficient biofilm attachment. Thus, a threshold probably exists for bacterial attachment and accumulation and if the biofilm thickness exceeds this threshold, crumbling and collapse occur. The present *in vitro* study experimented only the elementary physicochemical interaction phase of bacterial adhesion. In addition, only one *S. mutans* strain was tested, although the oral cavity is always implicated by many various microbial species. The effect of acquired pellicle, which can cover the physicochemical surface particulars of materials,[37] was not included in this study.

The main limitation of the current study was its inability to completely simulate the oral clinical setting (such as the storage of samples in the saliva). It has been showed that storage of samples in the saliva may alter or weaken the effect of HP by formation of a superficial protective layer of saliva on restoration surfaces and for bleached surfaces has a protective role.[2,9] Furthermore, evaluation of the effect of bleaching on surface free energy of different composite resins in the future studies can increase the reliability of the results.

CONCLUSION

Within the limitations of this study, the following conclusions can be drawn:

- Surface roughness of Filtek P90 silorane-based composite resin was not affected by different bleaching protocols
- Bleaching decreased the formation of biofilm on Filtek P90 silorane-based composite resin. The lowest amount of biofilm was noted in light-activated 35% HP group.

Financial support and sponsorship

Dental and Periodontal research center at Vice Chancellor for Research and Technology, Tabriz University of Medical Sciences.

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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