Original Article

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Effects of 40% hydrogen peroxide on the surface roughness and biofilm formation of three different types of resin composites

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Objective: This study aimed to examine the effects of hydrogen peroxide on three different resin composites (Z250: microhybrid, Z250XT: nanohybrid, and Z350XT: nanocomposite) in terms of surface roughness alteration and biofilm formation.

Materials and Methods: Forty-two samples were prepared from each material and then randomly divided into two groups for investigation. In total, 20 samples were used to determine the surface roughness, and the remaining 22 samples were used to determine biofilm formation. Finally, the samples were divided into two subgroups: the bleached group and the nonbleached group. In the bleached group, the samples were bleached with 40% hydrogen peroxide (opalescence boost). The bleaching procedures were conducted following the manufacturer's instructions. The surface roughness was assessed using an arithmetical mean height of an area (Sa) by a laser scanning microscope. For biofilm measurement, *S. mutans* was cultured on each sample coated with the acquired pellicle and stained with a Live/Dead Bac Light™ Bacterial Viability Kit. Biofilm formation was measured under a confocal laser scanning microscope.

Results: The surface roughness significantly differed among the three groups of materials without bleaching (p=0.000) and with bleaching (p=0.000). The roughness of Z250 was significantly greater than that of the other two samples, while no significant difference between Z350XT and Z250XT was observed. Compared with that of the samples without bleaching, the surface roughness of the three types of resin composites was significantly different (p<0.05). For biofilm formation, no significant differences among the groups were observed.

Conclusions: Bleaching affected the Sa of three different types of resin composites, but the change in Sa had no effect on the average volume of colonies at the substratum *of S. mutans* biofilms. The resin-based materials Z250, Z350XT and Z250XT represent potentially suitable materials for aesthetic restoration, and the 40% hydrogen peroxide bleaching agent had no adverse effects.

Keywords: biofilm formation, hydrogen peroxide, resin composites, S. mutans, surface roughness

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Introduction

Currently, tooth bleaching is commonly used to improve the self-esteem of patients who have nonaesthetic tooth colour [1]. Hydrogen peroxide is a commonly used bleaching agent because it effectively removes internal stains [2]. In addition, for patients with high aesthetic concerns who require restoration due to loss of tooth structure, resin composites are the material of choice for restorations that should be placed in highly aesthetic area [3].

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Among the currently used resin composites, microhybrid resin composites, nanocomposites, and nanohybrid composites are frequently employed [4]. These recently nanofilled composites have good mechanical strength and well-polished surfaces that maintain their integrity during long-term use, even in the posterior regions of the mouth, and their properties do not differ from those of microhybrid composites [5]. Previously, microhybrid composites were utilized for aesthetic restorations. Owing to the advancements in nanofilled composites, which possess superior mechanical qualities, the durability of polish retention and aesthetics, nanofilled composites are the most desirable type of resin composite [6].

The effect of bleaching agents on resin composites has been reported to increase surface roughness and biofilm formation on restorations, leading to restoration failure over time [7]. Conversely, few studies have reported a minor change in surface roughness among resin composites [8, 9]. However, current nanofilled composite resins have not yet been investigated.

The objectives of this study were 1) to examine the effects of hydrogen peroxide on three different resin composites in terms of surface roughness alteration and 2) to examine the effects of hydrogen peroxide on three different resin composites in terms of biofilm formation.

The hypotheses of this study were as follows: The null hypothesis for surface roughness is that there are no significant differences in surface roughness among the three different resin composites before and after bleaching with hydrogen peroxide. The null hypothesis for biofilm formation is that there are no significant differences in the biofilm formation of oral bacteria (*Streptococcus mutans*) among the three different resin composites before and after bleaching with hydrogen peroxide.

Materials and Methods

This study was approved by the Ethics Committee in Human Research (Faculty of Dentistry/Faculty of Pharmacy, Mahidol University Institutional Review Board; MU-DT/PY-IRB) with approval number 2023/044.1508.

Specimen preparation

The resin composites used in this study were microhybrid resin composites (Filtek Z250), nanocomposites (Filtek Z350XT) and nanohybrid resin composites (Filtek Z250XT). The details of the materials are shown in Table 1. The cylindrical shapes of the samples were prepared using plastic tubes with a diameter of 5 mm and height of 2 mm [10]. Each material was inserted into a plastic mould [10]. Each side of the plastic mould was covered with polyester celluloid strips (Stripmat, Polydentia, Mezzovico, Switzerland) and finally covered with a glass slide [10]. A light curing unit (LED Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein) with a light intensity of 1000 mW/cm^2 (measured by using Bluephase Meter II) was used to polymerize the sample close to it. The curing time of the sample was 20 s for both sides [10]. All the samples were kept at 100% relative humidity at 37 °C for 24 h [10]. The sample surfaces were polished with abrasive polishing discs (Sof-Lex extra thin, 3M ESPE, St. Paul, MN, USA) from coarse to superfine discs sequentially using a speed-controlled handpiece (TCM ENDO III, SybronEndo, Nouvag AG, Switzerland) under dry conditions with a speed control of 13,000 rpm [10]. For the polishing conditions, four different polishing directions were performed by one operator with seven polishing strokes for 15 s in each direction [10]. Stable pressure (approximately 2 N) was applied for polishing [10]. Each sample was rinsed with water and then air-blowed among each

polishing sequence [10]. After completing the 4 sequences of surface polishing, each sample was cleaned with distilled water (Faculty of Dentistry, Mahidol University, Bangkok, Thailand) with an ultrasonic cleaner (BioSonic UC125, Coltene Whaledent, Altstatten, Switzerland) for 5 min and followed by air blowing [10]. Each side of each sample was sterilized in a UV chamber for 45 min.

Sample size calculation: The required sample size was determined using G*Power version 3.1 (Heinrich Heine University, Düsseldorf, Germany) and the standard formula for comparing two independent means. Parameters were set at an $\alpha = 0.05$ (95% confidence level) and $\beta = 0.20$ (80% power), additional input parameters liked estimated variability (e.g., means and standard deviation) were from Wongprapatanata *et al.* [4]. Based on a standard deviation of 2.11 μm from the previous study and assuming a detectable difference (Δ) of 80% and 65% of σ, the calculated sample size was approximately 20 specimens per group.

Based on these calculations, a total of 42 specimens were prepared from each material, of which 20 were used for surface roughness testing and 22 for biofilm formation analysis. The samples in each group were then further divided into 2 equal subgroups to perform bleaching on half of the groups.

Table 1 Composition of the resin composites and bleaching products used in this study.

Material	Composition	Manufacturer
Resin based materials		
Filtek Z250 Type: Minifill microhybrid resin composite	- Matrix: Bis-GMA, UDMA and Bis-EMA - Inorganic filler: minifill and microfill - Average filler size: 0.6–1 μm and 40 nm - Filler type: Silica-zirconia	3M ESPE, St. Paul, MN, USA
Filtek Z350XT Type: Nanocomposite	- Matrix: Bis-GMA, UDMA, Bis-EMA and TEGDMA - Inorganic filler: nanofill - Average filler size: 5 – 100 nm - Filler type: Silica nanofillers, Zirconia-silica nanocluster	3M ESPE, St. Paul, MN, USA
Filtek Z250XT Type: Nanohybrid resin composite	- Matrix: Bis-GMA, UDMA, Bis-EMA, PEGDMA and TEGDMA - Inorganic filler: nanohybrid - Average filler size: 0.6 – 1 μm and 5 – 100 nm - Filler type: Silica-zirconia, Silica nanofillers, Zirconia-silica nanocluster	3M ESPE, St. Paul, MN, USA
Bleaching product		
Opalescence Boost	- 40% Hydrogen peroxide - Potassium nitrate - 0.11% Fluoride ion - Carbopol - Glycerine - Flavouring (pH=7)	Ultradent, South Jordan, USA

Bleaching Procedure for the Samples

The unbleached samples served as controls. The samples were placed in distilled water and stored at 37 °C for 2 weeks [11]. For the bleached group, the samples were bleached with 40% hydrogen peroxide (Opalescence Boost, Ultradent Products, South Jordan, USA). Two cycles were selected to represent a typical in-office bleaching protocol while limiting overexposure that could cause excessive surface degradation, as supported by previous study [11]. To simulate an in-office bleaching technique, 1 mL of 40% hydrogen peroxide bleaching gel (Opalescence Boost, Ultradent Products, South Jordan, USA) with approximately 1 mm thickness was gently applied to the specimen surface and left undisturbed (without active agitation) for 20 min. Before removing the bleaching gel, suction was used to aspirate most of the gel, and the specimen surface was then thoroughly rinsed with distilled water before a new layer of gel was reapplied for the second cycle.

Determination of surface roughness

Twenty samples of each material from both the bleached and unbleached groups were investigated for surface roughness using the arithmetic mean height of an area (Sa) with a laser scanning microscope (OLS5100 Laser Microscope, Olympus LEXT™, Tokyo, Japan) with a cut-off of 0.08 mm (Gaussian profile filter) at a magnification of 20X. Then, 3D images were constructed. One specimen was used to produce one record. The surface roughness parameter Sa was calculated using analysis software (LEXT, Olympus LEXT™, Tokyo, Japan). For standardization, the surface roughness was measured at the centre of each sample within an area of 3×3 mm^2 [11].

Determination of biofilm formation

Twenty-two samples of each material from both the bleached and unbleached groups were used to determine biofilm formation. For saliva-coated specimen preparation, unstimulated saliva was collected from 3 healthy individuals who had no medical problems or medicine intake within 1 month under protocols approved by the ethical committee. Unstimulated saliva was collected in the morning between 8:00–9:00 a.m. from three healthy volunteers. All volunteers refrained from eating or drinking for at least 1 h and did not brush their teeth for at least 1 h prior to collection to minimize confounding factors. None of the volunteers had any medical conditions or had taken medications within the past month. Five millilitres of saliva were centrifuged at 15000 × g at 4 °C for 15 min and then diluted in phosphate buffer solution (PBS) at a ratio of 1:10. The pooled diluted saliva was subsequently sterilized using a Millipore membrane with a pore size of 0.2 µm and then stored at 4 °C for no longer than 24 h until use. If storage is necessary for long periods, consider freezing the saliva at -80°C. To acquire pellicles, sterile samples were immersed in 1 ml of saliva and incubated at 37 °C for 16 h. Then, biofilm formation was carried out. For biofilm formation, the cariogenic bacteria *S. mutans* ATCC 25175 were cultured in brain heart infusion agar in a 5% CO_2 incubator (Forma CO_2 Incubators, Thermo Fisher Scientific, Waltham, MA, USA) at 37 °C for 48 h. Then, the bacteria were diluted in brain-heart infusion broth supplemented with 5% sucrose to achieve a cell density of 1×10^5 CFU/ml. Each saliva-coated sample was immersed in 1 ml of bacterial suspension, incubated in a 5% CO_2 incubator at 37 °C for 24 h, and then the samples were washed three times with 1 ml of distilled water [11]. For biofilm analysis, a sample from each group was randomly selected

to screen whether the biofilm formed using 0.1% crystal violet for 15 min, after which it was rinsed 2 times with PBS. The stained biofilms were observed under a stereomicroscope (Leica EZ4 HD, Leica Microsystems, Wetzlar, Germany) [11]. The quantity of *S. mutans* biofilms on 20 samples from each resin composite material, including both the bleached and unbleached groups, was analysed with a confocal laser scanning microscope (Leica DMi8, Leica Microsystems, Wetzlar, Germany) [11] using the Live/Dead Bac Light™ Bacterial Viability Kit (Molecular Probes, Eugene, USA), which is composed of two fluorescent dyes, namely, SYTO9 and isopropidium iodide. SYTO9- stains live bacteria with intact cell membranes and fluoresces green, whereas isopropidium iodide stains only dead bacteria with compromised membranes and fluoresces red. After that, the samples were observed using CLSM with optical lenses at a magnification of 20X and reconstructed into a 3D model using Leica LAS X software. At least three representative optical fields were examined for each sample. An excitation wavelength of 488 nm was used, and the emitted light was collected between 500 and 560 nm. Together with Leica LAS X software, COMSTAT2 (Technical University of Denmark, Kongens Lyngby, Denmark) was used to analyse the average volume (μm^3) of colonies at the substratum by the colour segmentation.

Statistical analysis

Statistical analysis was performed using PASW Statistic 18 (IBM, Armonk, NY, USA). The means and standard deviations of all the groups were calculated. The surface roughness and average volume of colonies at the substratum of materials and the effects of bleaching were analysed using two-way ANOVA.

To identify the differences in surface roughness among the experimental groups, analysis of variance and multiple comparisons were performed with respect to the independent factors (materials and bleaching), separately. The Scheffe test was used to test the differences within materials, and the independent t-test was used to test the difference between the bleached and unbleached groups. Statistical analysis was performed with a *p*-value of 0.05, indicating statistical significance.

Results

Determination of surface roughness

All surface roughness (Sa) values obtained before bleaching for Z250(0.3031), Z350X(0.2365), and Z250XT(0.2459), and those after bleaching for Z250(0.3355), Z350XT(0.2721), and Z250XT(0.2883) that were below the clinically relevant threshold of 0.6 µm, as previously recommended for acceptable surface smoothness [10].

The surface roughness of three resin composite materials, i.e., Z250, Z350XT, and Z250XT, in both the unbleached and bleached groups was determined. Two-way ANOVA revealed a significant effect of different resin composites ($p<0.001$) and a significant effect of bleaching ($p<0.001$) without interaction between the types of materials and bleaching ($p=0.687$). For multiple comparisons, Scheffe's test indicated that in both the bleached and unbleached groups, the roughness of Z250 was significantly greater than that of Z350XT and Z250XT ($p<0.001$), whereas no significant difference was noted between Z350XT and Z250XT. ($p>0.05$).

Table 2 Means and standard deviations in μm of surface roughness (Sa) of three resin composite materials subjected to unbleached and bleached treatments.

Types of resin composite	Treatment	
	Unbleached	Bleached
Z250	$0.3031 \pm 0.02672^{a, B}$	$0.3355 \pm 0.03551^{a, A}$
Z350XT	$0.2365 \pm 0.02965^{b, B}$	$0.2721 \pm 0.02133^{b, A}$
Z250XT	$0.2459 \pm 0.04451^{b, B}$	$0.2883 \pm 0.03465^{b, A}$
p-value	< 0.001	< 0.001

Note: The same small superscript letter indicates no statistically significant difference within the same columns, whereas the same capital superscript letter indicates no statistically significant difference within the same rolls ($p < 0.05$).

Representative 3D images of the surface topography of the resin composite materials observed under a laser microscope at 20X magnification are shown in Figure 1. Relatively smooth surfaces of Z350XT and Z250XT were observed in both the bleached and unbleached groups. More surface irregularities were observed in both the bleached and unbleached groups of Z250 than in those of Z350XT and Z250XT.

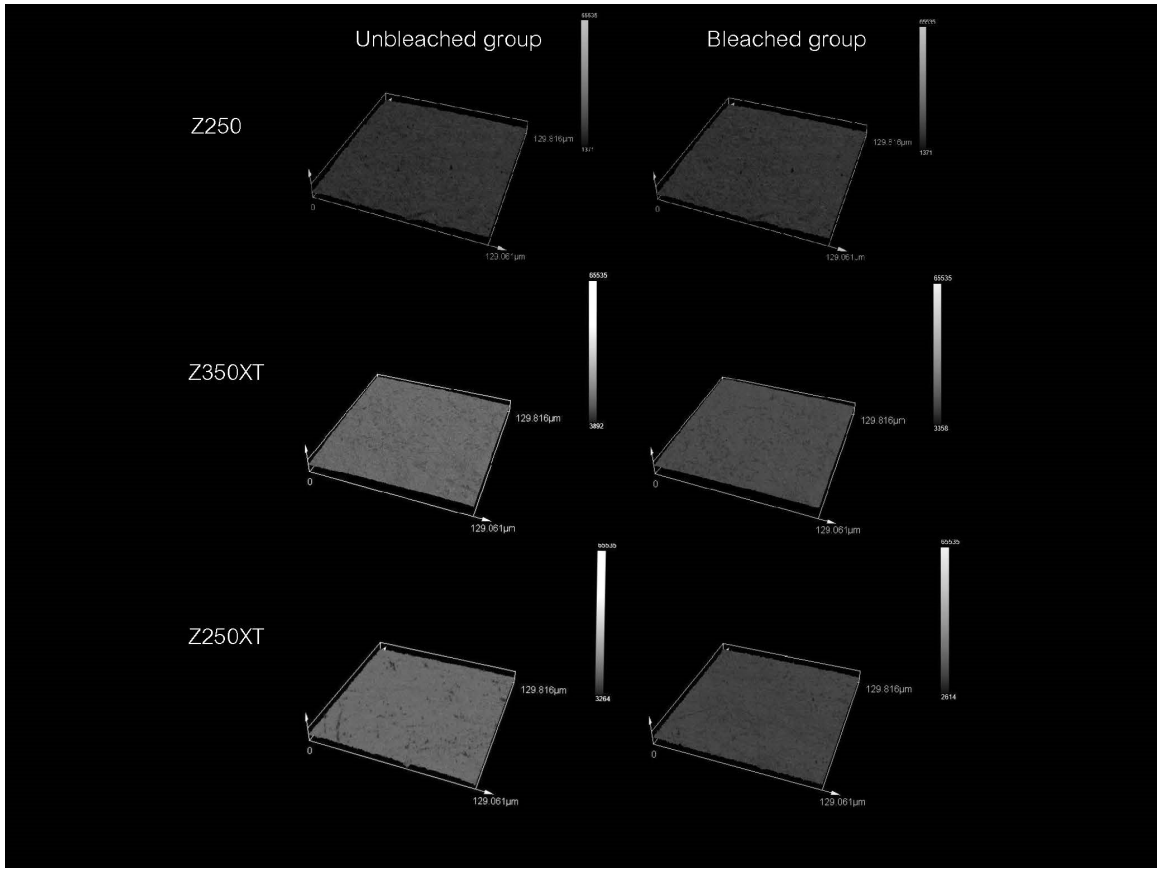

Figure 1 Representative 3D images of the surface topography of all treatment groups observed under a laser scanning microscope at 100X magnification.

The color in a 3D profilometer profile involves analyzing the surface characteristics of a specimen based on surface roughness. Colors are used to represent different heights of the surface relative to a reference plane. The color gradient often varies from blue (or a darker color) for lower heights to red (or a brighter color) for higher heights. Green represents the reference plane or zero height. Blue indicates areas that are below the reference plane (negative heights). Red demonstrates areas that are above the reference plane (positive heights).

The 3D images illustrate the surface topography of the resin composites before and after bleaching. The colour scale bars indicate surface height variations, where blue to dark blue represents valleys or low roughness values (approximately –20 to –8 µm), green indicates intermediate areas (~0 µm), and yellow to red represents peaks or higher roughness values (up to +20 µm). Unbleached Z250 showed relatively uniform green surfaces, while bleached Z250 exhibited slightly more yellow–green regions. Z350XT and Z250XT specimens demonstrated predominantly blue–green colours, with the bleached Z250XT displaying the widest height variation (–10.905 to +19.585 µm).

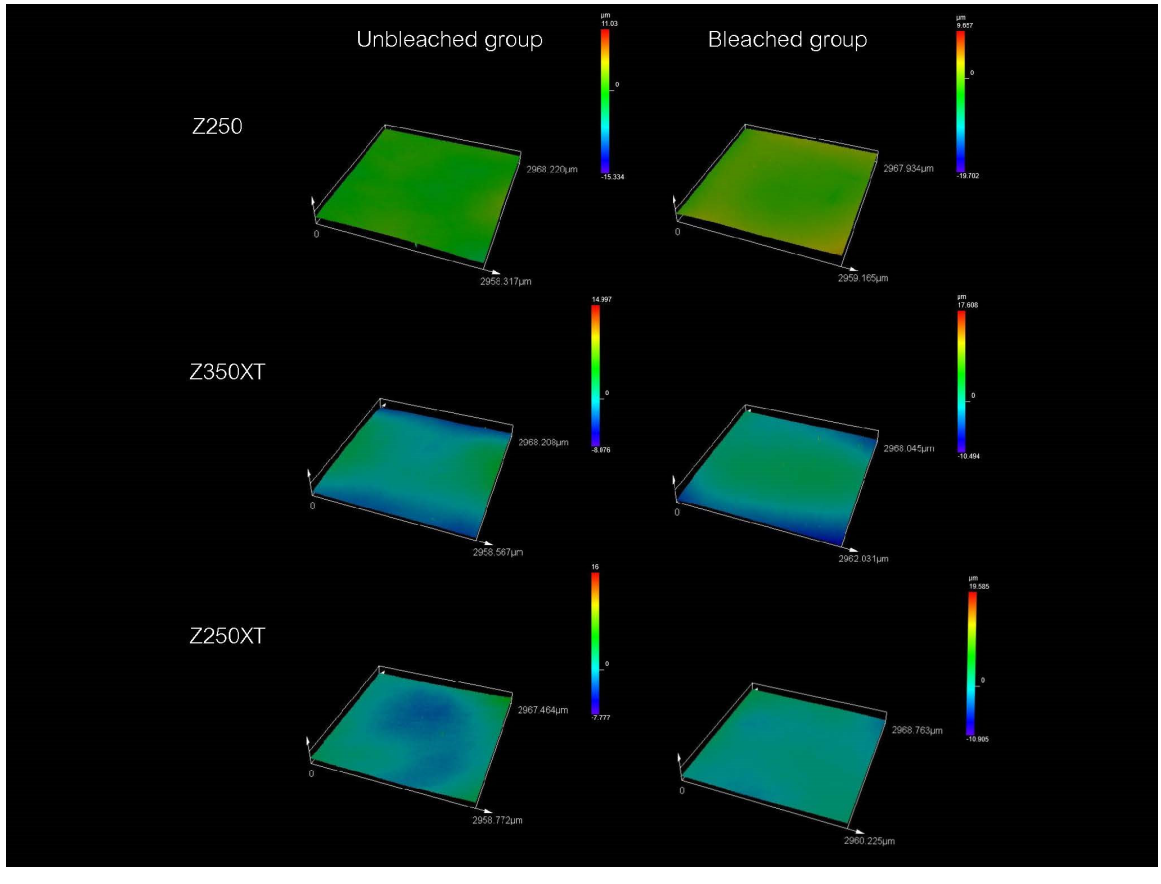

Figure 2 Representative 3D images of the surface roughness values of all the treatment groups observed under a laser scanning microscope at 20X magnification.

Determination of biofilm formation

The effects of the type of resin composite (p=0.258), bleaching (p=0.841), and interaction between materials and bleaching (p=0.769) on the average volume of *S. mutans* biofilms were revealed with two-way ANOVA. There were no statistically significant effects on the average volume of bacterial colonies at the substratum. The means and standard deviations of the average volume of colonies at the substratum of the three resin composite materials in both the unbleached and bleached groups are shown in Table 3. The average volume of *S. mutans* colonies at the substratum of the unbleached groups ranged from 3874696.130 ± 3263709.365 to 4804371.613 ± 2349383.537 μm^3, whereas that of the bleached groups ranged from 4271074.735 ± 3046595.476 to 4968685.248 ± 2747608.698 μm^3.

Representative 2D and 3D images of the average volume of colonies at the substratum at 20X magnification are shown in Figure 3 and Figure 4, respectively. The colour intensity of the *S. mutans* biofilms did not differ among the sample groups. The 2D and 3D images clearly revealed a more greenish colour of live (A) than reddish colour of dead cells (B), resulting in a relatively greenish colour (C) in the combination of live/dead cells for all groups.

Table 3 Means and standard deviations of the average volume of *S. mutans* colonies at the substratum (μm^3) of three resin composite materials subjected to the unbleached and bleached treatments.

Types of resin composite	Treatment	
	Unbleached	Bleached
Z250	4804371.613 ± 2349383.537	4968685.248 ± 2747608.698
Z350XT	4760803.733 ± 2324684.946	4446437.841 ± 2567428.117
Z250XT	3874696.130 ± 3263709.365	4271074.735 ± 3046595.476

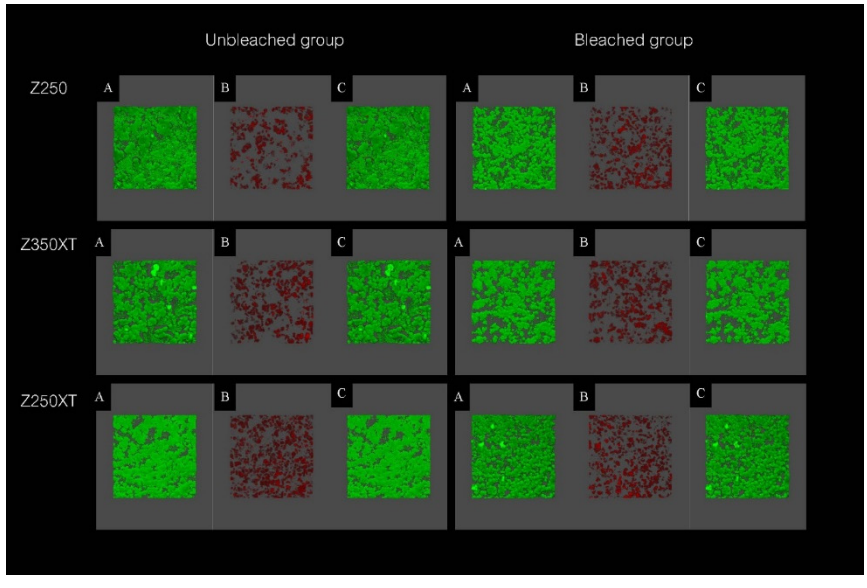

Figure 3 Representative 2D images of the average volume of colonies at the substratum of all groups were obtained using confocal laser scanning microscopy and Leica LAS X software at 20X magnification. A) Live cells are shown in green; B) dead cells are shown in red; C) the combination of live and dead cells.

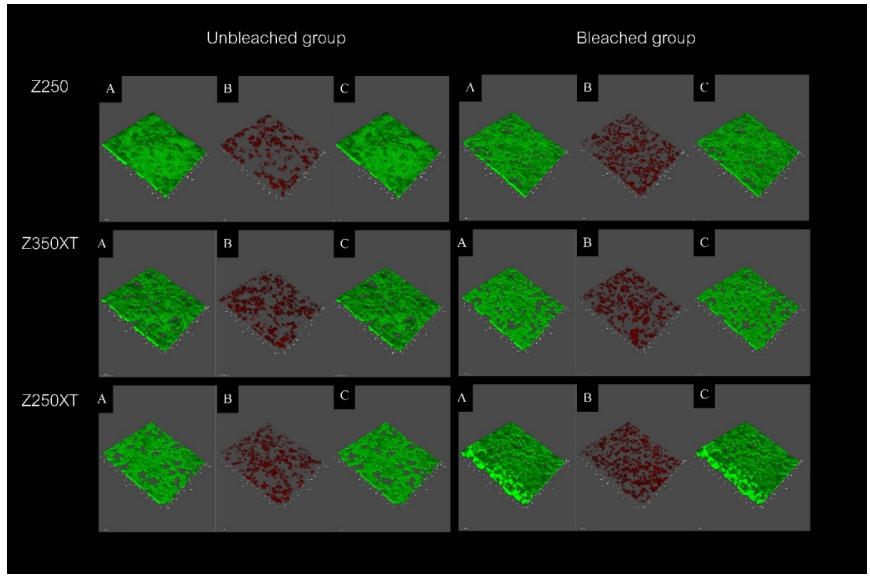

Figure 4 Representative 3D images of the average volume of colonies at the substratum of all experimental groups were observed under a confocal laser scanning microscope and analysed using Leica LAS X software at 20X magnification. A) Live bacterial cells are shown in green; B) dead bacterial cells are shown in red; C) superimposed live and dead cells.

Discussion

Bleaching with hydrogen peroxide significantly increased the surface roughness of all tested composites. Z250 showed the highest roughness values, while Z350XT and Z250XT remained smoother under both bleached and unbleached conditions. These results confirm that filler size and composition influence bleaching-induced surface changes. Comparable outcomes were achieved in the bleached categories, with Z350XT and Z250XT maintaining notably lower surface roughness than Z250. Based on the 3D surface topography results, Z250XT and Z350XT presented largely undamaged, smoother surfaces, whereas Z250 exhibited more pronounced irregularities.

The maximum surface roughness value of Z250 in both the bleached and unbleached conditions corroborated the findings of the previous study, which indicated a correlation of surface roughness with the larger particle size of Z250 [12]. Furthermore, the smoothness of nanohybrid composites, in comparison with that of microhybrid composites, is influenced by the size of the filler when exposed to greater concentrations of hydrogen peroxide [13]. The reduced surface roughness of Z250XT in comparison with that of Z250 may be attributed to this phenomenon. The Z350XT nanocomposite exhibited the lowest surface roughness rating because of the presence of submicron-sized filler particles [14].

Z250 is a microhybrid composite containing larger silica–zirconia filler particles (0.6–1 μm) that are more prone to protrusion or plucking when the resin matrix is degraded by hydrogen peroxide. In contrast, Z350XT (nanocomposite) and Z250XT (nanohybrid) contain uniformly distributed nanofillers and zirconia–silica nanoclusters (5–100 nm), which provide stronger filler–matrix bonding and greater resistance to bleaching-induced surface alterations [6, 15-19].

Surface roughness modification is a detrimental consequence of the use of bleaching chemicals. The extent of changes may differ depending on the composition of the resin materials, the concentration of the bleaching gel, and the method of exposure [20]. It was postulated that the use of oxidizing agents might either increase the porosity of the polymer matrix or cause the filler to detach due to an increase in water absorption, resulting in alterations in surface roughness and gloss. Peroxides trigger the oxidative breakage of polymer chains, particularly those that target unreacted double bonds, which are the most susceptible areas of polymers. This process explains how bleaching agents modify the surface roughness of resin composites [21]. Bleached composites may absorb more water because hydrogen peroxide can oxidatively cleave polymer chains, creating microcracks and increasing surface porosity. This structural degradation weakens the filler–matrix interface, allowing greater water penetration and filler debonding under bleaching conditions [21,23–25]. Moreover, the absorption of water can lead to stress corrosion and the separation of fillers, ultimately increasing the roughness of the composite resin surface [23]. Furthermore, the dimensions of the filler particles play crucial roles in determining the surface roughness and polishing ability of restorative materials [24]. Dimensions specifically refers to the filler particle size and its distribution. Larger fillers (≈0.6–1 μm) found in microhybrid composites like Z250 are more prone to protrusion or plucking during bleaching, increasing surface irregularities, whereas nanosized fillers (< 100 nm) in Z350XT and Z250XT create a denser filler–matrix network and smoother surfaces after bleaching [6,15-16].

Because of its higher organic matrix content and smaller filler quantity, microparticulate resin is more vulnerable to the erosive effects caused by bleaching chemicals [25]. The surface roughness may be influenced by the concentration of hydrogen peroxide and the duration of its application. A prior investigation indicated that concentrations of hydrogen peroxide ranging from 30% to 35% had an impact on the surface roughness of composite resins [26]. This study revealed that the bleaching agent with 40% hydrogen peroxide altered the surface roughness of the tested materials. Taken together, owing to its filler sizes in both the unbleached and bleached groups, the microhybrid composite exhibited greater surface roughness compared with the other two groups.

Previous studies have reported that increased surface roughness can promote biofilm development on restorative materials [12, 27–29]. This is consistent with the present findings showing that, although bleaching did not significantly increase *S. mutans* biofilm volume on any material, Z250, which exhibited the highest roughness, showed slightly greater bacterial retention trends. The rougher surface may provide more retention sites for initial bacterial adhesion and protect microorganisms from shear forces. However, some authors have argued that surface roughness alone may not always correlate with biofilm accumulation, suggesting that other factors such as salivary pellicle formation, bacterial species, and surface free energy also influence adherence [30–33].

These conflicting results indicate that while surface roughness is an important parameter, it should be interpreted together with other surface and environmental factors when evaluating the risk of biofilm-related restoration failure.

Hence, the second part of this study aimed to assess the production of *S. mutans* biofilms on three different types of resin composites with and without bleaching. Regarding the relationship

between surface roughness and the risk of biofilm formation, restorative materials with a surface roughness above the tolerable threshold of 0.2 μm presented a greater risk of plaque build-up, gingival irritation, and dental cavity appearance [34]. Our results revealed that all three materials in both the unbleached and the bleached groups had Sa values greater than 0.2 μm, and *S. mutans* biofilms were detected on all the materials. In addition, a previous study examined the relationship between surface roughness and biofilm formation. This study demonstrated that samples with surface roughness values ranging from 0.6 to 0.9 μm presented greater biofilm formation levels than samples with surface roughness values less than 0.6 μm [10]. Our study revealed that the surface roughness values of both unbleached and bleached resin-based composites were less than 0.6 μm. This finding can be attributed to the fact that although the surface roughness of the three distinct types of resin composite varied and increased after bleaching, it did not have any effect on the amount of *S. mutans* biofilm [10]. When the 2D and 3D images of biofilms were examined, there was no noticeable difference in biofilm thickness between the unbleached and bleached groups of the resin-based material.

Regarding the bioactivity of the materials, all the groups did not exhibit any differences in antibacterial properties. Additionally, there were no noticeable differences in the ratio of live to dead bacterial cells among the different groups. Because all the tested materials were from the same manufacturer, they should have almost the same compositions that presented the same bioactivity.

The bleaching procedure and biofilm formation were tested under laboratory conditions, which do not fully replicate the dynamic oral environment. Therefore, the application should be clinically concerned.

Only three composites from a single manufacturer and one concentration of hydrogen peroxide (40%) were evaluated. Other brands, filler technologies, or lower concentration bleaching products may produce different results. In addition, biofilm assessment was performed using only Streptococcus mutans. Oral biofilms in vivo are polymicrobial, and interactions among species could influence biofilm formation.

For further study, a broader material spectrum, including various brands, filler systems, and different bleaching agents or concentrations, may be necessary to determine generalizability. Moreover, studying biofilm formation with multispecies oral microbiota or in situ models may provide a better simulation of clinical scenarios.

Conclusion

Within the limitations of this study, bleaching with 40% hydrogen peroxide increased the surface roughness of the three tested resin composites (Filtek Z250, Filtek Z350XT, and Filtek Z250XT) to different extents. However, this change in roughness did not significantly influence *S. mutans* biofilm volume under the specific experimental conditions.

Clinical relevance

Under the conditions tested in this study, the three investigated resin composites maintained acceptable surface roughness regarding to *S. mutans* biofilm formation after bleaching with 40% hydrogen peroxide. These findings suggest that, within these specific parameters, bleaching may not compromise the tested composites' surface integrity, but broader clinical implications should be interpreted with caution.

Conflicts of Interest

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

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