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Effect of laser bleaching, ultrasonic scaling and powered tooth brushing on surface roughness and bacterial adherence of class V composite restorations

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ABSTRACT

Objective: To evaluate and compare the effect of diode laser assisted bleaching, ultrasonic scaling and powered tooth brushing on surface roughness and bacterial adherence on class V cavities restored with composites.

Materials and methods: A total of one hundred and twenty samples (40 samples each of Brilliant Everglow, Beautifil II and Heytec-N) were prepared in standardized stainless steel molds. The samples were further subdivided into four subgroups i.e. one control group (without any intervention) and three experimental groups - diode laser assisted bleaching, ultrasonic scaling and powered tooth brushing consisting of 10 sample each. Surface roughness was measured quantitatively with the help of 3D Optical Profilometer. For bacterial adherence analysis *S. mutans* strain (ATCC 25175) was cultured in BHI medium and samples were evaluated for the presence of viable bacteria using the Colony Forming Unit (CFU) count. Results obtained were then tabulated and subjected to statistical analysis.

Results: Diode laser bleaching caused a significant increase in surface roughness and bacterial adherence with lowest mean change exhibited by Heytec-N followed by Beautifil II and highest by Brilliant Everglow group. Similarly, Ultrasonic scaling increased the surface roughness of all the three tested samples with significant difference between the groups. Powered tooth brushing had no effect on the surface roughness and bacterial adherence of the tested composites.

Conclusion: Diode assisted laser bleaching and ultrasonic caused significantly higher surface roughness and bacterial adherence values for all the tested composites. It may therefore be recommended to do finishing and polishing of restorations after such procedures.

1. Introduction

Class V restorations have the highest failure rates in adhesive dentistry. Factors such as stress distribution pattern, choice of restorative material, surface finish and margins of these restorations determine clinical success.[1,2]

A variety of class V cavity filling materials are available with composite restorations being in high demand because of favorable mechanical properties and high-quality aesthetic results.[3] New materials with surface reacting glass ionomer filler particles and composites with antibacterial properties have been introduced for better outcomes.[4]

Surface quality is the principal factor for determining clinical success. Smooth surface reduces plaque accumulation and staining resulting in improved clinical performance of class V restorations.[5] Routine oral healthcare procedures and treatments such as laser assisted bleaching, ultrasonic scaling and powered tooth brushing affect the integrity of the surface and also surface finish of these restorations that may provide greater surface area for bacterial adhesion and their multiplication.[6]

In-office laser bleaching is a clinician friendly procedure but it may have deleterious effects on restorative materials in patients who are already having restorations or have carious defects that require restorations prior to whitening treatment.[7] Ultrasonic scaling used to remove deposits of plaque and calculus predominantly around the gingival third aspect of the teeth may also have an effect on the surface finish of class V composite restorations as these deposits can make it difficult for the

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dentist to distinguish between the tooth and tooth colored restorations.[8] Tooth brushing can be another source of mechanical breakdown of the composite-tooth interface.[9]

As per our knowledge, this is the first study that has evaluated the effect of treatment and routine procedures on surface finish and bacterial adherence of composites. Thus, the present study was undertaken to evaluate and compare the effect of diode laser assisted bleaching, ultrasonic scaling and powered tooth brushing on surface roughness and microbial adherence of newer class V restorative materials. The first null hypothesis tested was that there was no difference in change in surface roughness and bacterial adherence of composites studied (Brilliant Everglow, Beautifil II, Heytec-N) after diode laser assisted bleaching, ultrasonic scaling and powered tooth brushing and secondly that there was no difference in the effect of different surface treatments on surface roughness of composites studied and subsequent bacterial adhesion.

1.1. Materials and methods

1.1.1. Preparation of samples

Institutional ethical committee approval was obtained prior to the commencement of the research. Sample size was calculated using:

$$n = \frac{(\sigma 1^2 + \sigma 2^2)(Z_{1-\alpha/2} + Z_{1-\beta})^2}{\Delta^2}$$

The notations are as below:
n = sample size of Groups
σ_1 = standard deviation for Group I = 0.051
σ_2 = standard deviation for Group II = 0.057.
Δ = difference in group means = 0.069.
$Z_{1-\alpha/2}$ = two-sided Z value (eg. Z = 1.96 for 95% confidence interval).
$Z_{1-\beta}$ = power = 80%

It was revealed from the results of the pilot study done on four samples of each subgroup from the three groups, that the expected Standard Deviation and mean difference of parameter of Group 1 & Group 2 was 0.051and 0.057 respectively and mean difference was 0.069 of two groups for variables. On using the formula on the Open Epi, Version 3 software, we found the sample size for each subgroup group to be 10, Hence a total sample size of 120 for all the three groups was taken.

Three composites were used in this study: a universal submicron hybrid composite (Brilliant Everglow, Coltene/Whaledent AG), a universal nanohybrid composite containing s-PRG ionomer particles (Beautifil II, Shofu, Japan) and a nanohybrid composite (Heytec-N, Heydent, Germany). A total of one hundred and twenty samples were made, forty samples per composite. Samples were prepared in standardized stainless steel molds (10 × 2mm). Composite was packed against mylar strip and then was polymerized from both sides through1mm thick glass slide with LED light (Bluephase-N, IvoclarVivadent,USA) as per manufacturer's instructions (Fig. 1). After removal from mould, samples were kept in distilled water for 6 days at 37 °C under light-proof conditions. To reduce the amount of residual monomer that may leach out during the subsequent incubation, each sample was rinsed twice a day with 1 ml of distilled water.[10]

2. Grouping of samples

The samples of each composite were further subdivided into four subgroups randomly i.e. one control and three experimental of 10 samples each (Fig. 2).

3. Part- a: surface treatments

3.1. Control group (subgroup A)

Samples were not subjected to any treatment.

3.2. Diode laser assisted bleaching (subgroup B)

Prior to activation, disc surfaces were covered with Laser Smile gel (Biolase, Technology, Inc, San Clemente, Calif.) comprising of 35% hydrogen peroxide to a standardized thickness of 1 mm.Gel was then activated by diode laser (Biolase EpicX Diode laser, India, λ = 980 nm) according to the manufacturer's recommendations. Subsequently bleaching gel was rinsed using distilled water for 30 s.

3.3. Ultrasonic scaling (subgroup C)

Ultrasonic scaling was performed on all discs for 60 s under copious

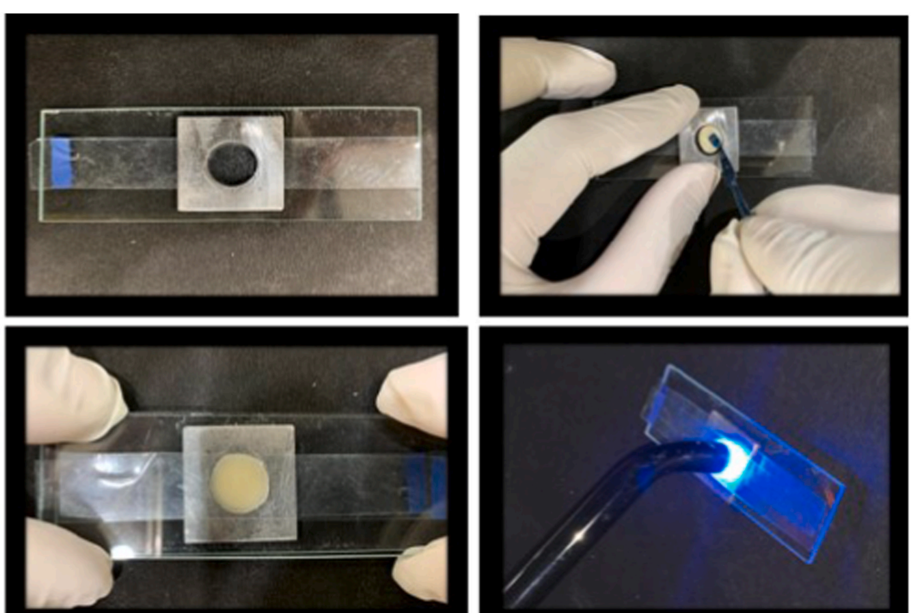

Fig. 1. Figure depicting preparation of samples A. Placement of mould B. Placement of restorative material into the mould C. Placement of matrix strip and glass slide over the mould D. Light curing of composite by LED light.

Fig. 2. Grouping of specimens into three experimental groups: Group 1: Brilliant Everglow, Group II: Beautifil II and Group III: Hytec-N.

water flow at power setting of 5 with ultrasonic scaler (Acteon Satelec P5 booster scaler, Acteon group ltd. UK). The tip of the scaler was angled approximately $15^{o}$with respect to the surface of the composite disc.

3.4. *Powered toothbrushing (subgroup D)*

Specimens were subjected to powered tooth brushing with the help of a customized tooth brushing simulating machine. A slurry of toothpaste (Colgate-Palmolive, India) and artificial saliva (Wet mouth, ICPA health products ltd.,India) was made and used to coat experimental discs. Coated discs were then placed on the working platform of toothbrushing simulating machine. Discs were brushed for 2 h to simulate 7 years.

4. Quantitative surface roughness evaluation

A 3D Optical Profilometer (Zeta instruments, KLA Company, USA) attached to a image software was used to assess the surface roughness. Based on the peaks and valleys seen in the area under analysis utilising a profilometer with a 0.8 mm cut off and 2.4 mm assessment length, the software used to create the photographs provided arithmetic roughness mean (Ra) data. This led to the creation of a 3D picture of the specimen surface profile. After that, each specimen's side and middle 10 mm 10 mm portions received three 3D photographs, the mean of which was taken.Results obtained were then tabulated and subjected to statistical analysis.

5. PART-B: bacterial adherence analysis

5.1. *Culturing of Streptococcus mutans strain and incubation of samples*

S. mutans strain (ATCC 25175) was cultured in brain heart infusion medium (BHI). Firstly, samples were incubated in distilled water for a duration of 24 h. Then they were transferred to 24-well UV sterilized cell culture plates. After that, 1 ml of bacteria suspension having optical density of DO600= (0.6–0.7), assessed using Spectrophotometer, was added to the surfaces of the tested samples and incubated in aerobic conditions for 96 h at 37 °C.

5.2. *Colony-forming unit counts*

To determine the number of CFU counts after incubation, the samples were drained and subsequently transferred into sterile tubes with 1 ml of fresh BHI. Tubes were then whirled for 1 min at 2400 rpm in a vortex mixer (Scientech Technologies Ltd., Indore, India). Bacteria that got detached were sequently diluted and further inoculated on BHI agar plates. Plates were aerobically incubated at 37 °C for 96 h. Finally, colony count was done manually with the help of bacterial colony counter (Simtronics Digital Colony Counter, New Jersey, USA) and results were tabulated in CFU/10^{-2}ml.

6. Statistical analysis

Statistical analysis was carried out SPSS 20 software. Descriptive statistics of mean ± S.D. of all parameter were calculated for all variables. The normality of the data was tested by Shapiro Wilk test and the data was found to be normal. One Way ANOVA Test was used to assess the differences in surface roughness and bacterial adherence values before and after treatment. The differences in mean surface roughness and associated bacterial adherence was evaluated with post hoc Bonferroni test. Correlation between surface roughness and bacterial adherence was evaluated with Pearson's Correlation Coefficient. The Level of significance was set at 5%.

6.1. Results

The results of the quantitative analysis was recorded and is represented in Figs. 3 and 4. **For Control group**, each restorative material had the lowest mean surface roughness and bacterial adherence values as compared to treatment groups with highest value shown by Brilliant Everglow and insignificant difference between Beautiful II and Heytec-N composites. **In diode laser assisted bleaching group**, all the composites showed statistically significant increase in surface roughness as compared to control group. The lowest value of mean change in surface roughness as well as bacterial adherence was exhibited by Heytec-N followed by Beautifil II and highest was observed in Brilliant Everglow group. **In ultrasonic scaling group**, there was increased surface roughness and bacterial adherence of all the three tested samples as compared to control group with statistically significant mean change in surface roughness between the three. **In powered tooth brushing group**, although the surface roughness increased in all the three composites as compared to control group but the increase was insignificant. Also, all the three composites Beautifil II, Heytec- N and Brilliant Everglow had statistically insignificant values of mean difference in surface roughness (Table 1).

Surface roughness and bacterial adhesion showed a similar trend irrespective of the composite and the surface treatment employed and reported significant positive correlation (Table 2) ($p < 0.05$).

6.2. Discussion

The restorative material may respond in a different manner when subjected to various dental procedures like bleaching or scaling and to routinely done practice of tooth brushing. Such treatments may alter the surface properties of composite which in turn might influence bacterial colonization and moreover, the clinical performance of the restoration.[11]

In our study, surface roughness and bacterial adherence of all the three tested composites significantly increased after diode laser assisted bleaching and ultrasonic scaling but the change was insignificant in powered tooth brushing group, so the first part of the null hypothesis was partially rejected. Out of all the three modalities ultrasonic scaling had the most deleterious effect on the composites studied. Secondly, all the different surface treatments presented different effects on the composites studied with Brilliant Everglow composite performing worst in diode laser assisted bleaching and ultrasonic scaling group.Consequently, the second part of the null hypothesis was partially rejected.

In the Control Group, all the three restorative materials evaluated reported low mean surface roughness values (<0.2 μm) which according to Bolen et al.[12] is considered to be clinically acceptable. A significant positive correlation was reported between surface roughness and

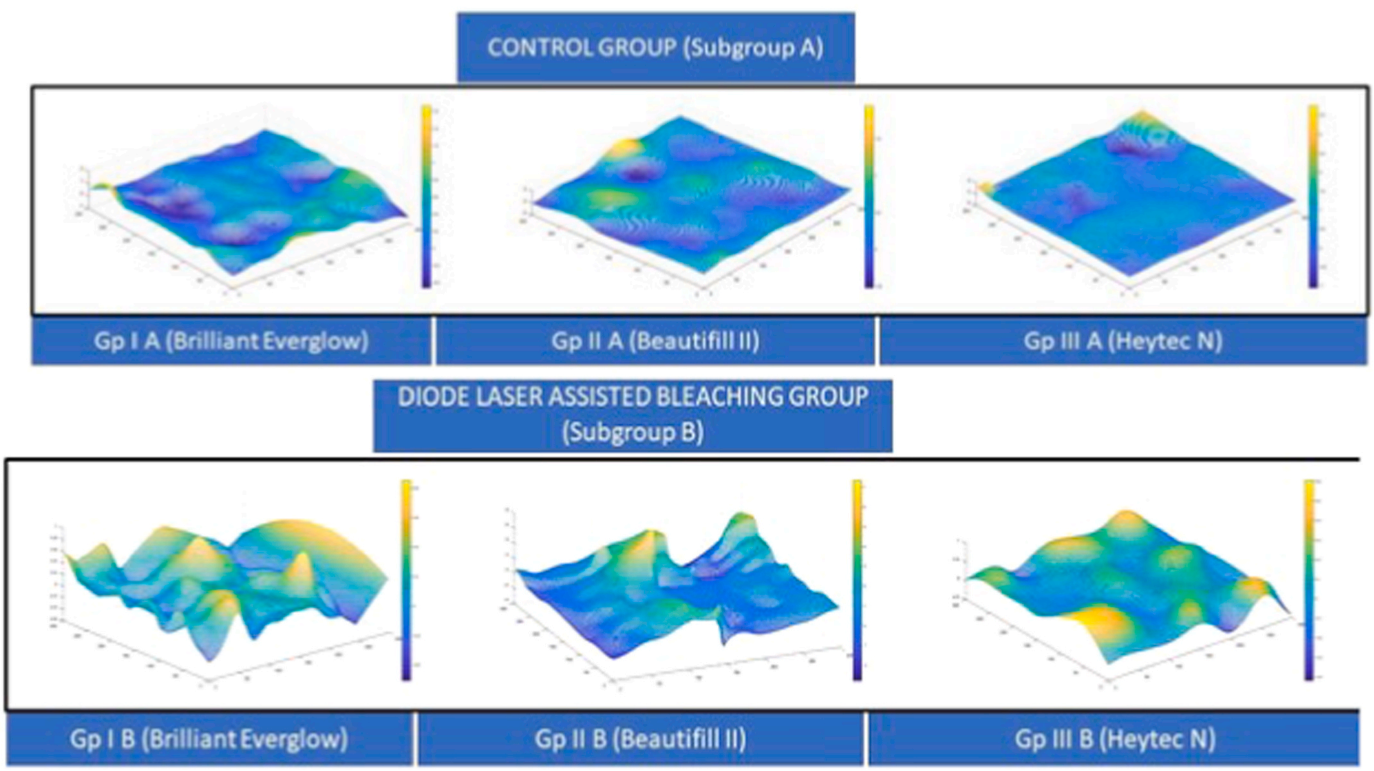

Fig. 3. 3D Profilmetric images images of control group (Subgroup A) and Doide Assisted Laser Bleaching Group (Subgroup B) for the three groups (Group I: Brilliant Everglow, Group II: Beautifill II, Group III: Hytec-N).

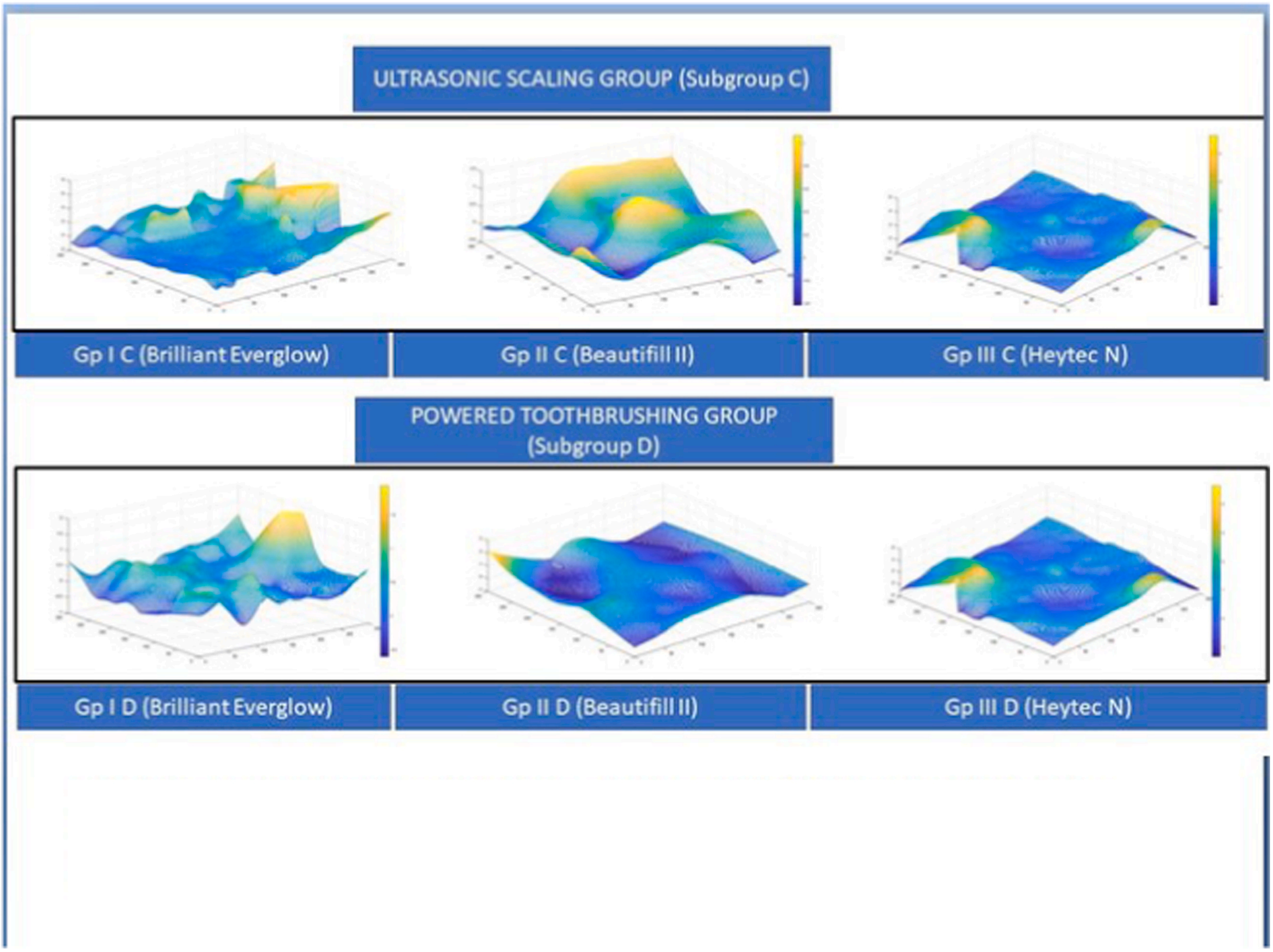

Fig. 4. 3D Profilmetric images of Ultrasonic Group (Subgroup C) and Diode Powered ToothBrushing Group (Subgroup D) for the three groups (Group I: Brilliant Everglow, Group II: Beautifill II, Group III: Hytec-N).

bacterial adhesion. Rough surface increases the surface area available for adhesion by a factor of 2–3.and thereby promoting bacterial adherence.[12] Minor surface imperfections within the polyester matrix may be reproduced in the composite samples and therefore, 100% perfect samples were not obtained.[12] Amongst them, Beautifil II and Heytec N showed lowest baseline mean surface roughness as both are nanohybrid composites with filler content of 83 wt% and 82 wt% respectively. On the other hand, Brilliant Everglow, a submicron hybrid composite with filler content of 74 wt% showed significantly greater surface roughness values.[13,14] Bacterial adherence was reported among the samples which could be attributed to the lack of salivary simulation and mechanical removal by oral hygiene measures in invitro setups.

Surface roughness and consequent bacterial adherence increased significantly in **diode laser assisted bleaching group** as compared to control group. These findings can be corroborated to the studies by **Wongprapratna et al.,**[15] **Markovic et al.**[16] and **Rattacaso et al.**[17] Bleaching agents affect the interface between the resin matrix and the filler-matrix by causing cleavage of the polymer chains induced by the free radicals.[18] Also, the oxidizing effect of the peroxide causes alteration in the surface morphology in the polymer matrix of the resin based material.[19] The lowest value of mean change in surface roughness and bacterial adherence was exhibited by Heytec-N followed by Beautifil II and highest was observed in Brilliant Everglow group. This could possibly be attributed to the high filler loading(82 wt%) and low organic matrix content in Heytec N compared to Brilliant Everglow which have high organic matrix content and low filler loading (76 wt%). Inspite of comparable filler loading, Heytec-N performed superior compared to Beautifil II which could be justified by the difference in organic matrix which is UDMA (high degree of conversion)in Heytec N whereas Beautifil II has Bis-GMA(low degree of conversion) and a small percentage of TEGDMA.[20]

Ultrasonic scaling caused significant increase in surface roughness for all the three composites with statistically significant differences between them which is in accordance with the study by **Hegde et al.**[21]The difference in the size and content of filler particles and the characteristics of the organic matrix could account for the differences in the roughness values. The mean surface roughness was highest and statistically significant for the Brilliant Everglow group followed by Heytec-N and Beautifill II. Brilliant Everglow has larger sized filler particles and reduced filler loading that could result in higher roughness values. Heytec-N contains a softer organic matrix (UDMA) that may be removed during the ultrasonic procedure resulting in a rough surface.[22] Beautifil II contains s-PRG filler particles in which the glass core is enveloped by a hydrogel. The outer core may be favourably removed during the ultrasonic scaling causing the protrusion of the harder glass surface thereby, increasing the surface roughness.[23,24]

In **powered toothbrushing group**, although the surface roughness and bacterial adherence increased in all the three composites as compared to control group, the increase was insignificant. The toothpaste used during tooth brushing may act as a mild abrasive that was

Table 1
Surface roughness (μm) and bacterial adherence values (CFU/10^{-2}ml) of Group I (Brilliant Everglow), Group II (Beautifill II) and Group III (Heytec-N) for Control group (Subgroup-A) Diode laser assisted bleaching group (Subgroup- B)Ultrasonic scaling group(Subgroup- C) and Powered toothbrushing group (Subgroup- D).

Group number	Group name	Surface roughnessvalues Ra (μm)				Bacterial adherence values (CFU/10^{-2}ml)			
		Control group (Subgroup-A)	Diode laser assisted bleaching (Subgroup- B)	Ultrasonic scaling (Subgroup- C)	Powered toothbrushing (Subgroup- D)	Control group (Subgroup-A)	Diode laser assisted bleaching (Subgroup- B)	Ultrasonic scaling (Subgroup- C)	Powered toothbrushing (Subgroup- D)
Group I	Brilliant Everglow	0.1651 ± 0.0475	0.3977 ± 0.0568	0.3118 ± 0.0522	0.1870 ± 0.0695	132.70 ± 22.549	331.40 ± 23.987	270.2270.21 ± 23.87	140.31 ± 33.589
Group II	Beautifill II	0.0968 ± 0.0452	0.3070 ± 0.0507	0.2507 ± 0.0400	0.1519 ± 0.0244	90.20 ± 21.893	291.40 ± 24.514	228228.01 ± 21.807	102.01 ± 21.741
Group III	Heytec-N	0.0769 ± 0.0343	0.2388 ± 0.0502	0.1882 ± 0.0298	0.1489 ± 0.0261	95.30 ± 28.15	231.87 ± 48.645	194.70 ± 21.471	98.81 ± 30.188
P value									
Group I vs Group II	Brilliant Everglow vs Beautifill II	0.004*	0.002*	0.009*	0.007*	0.002*	0.006*	0.001*	0.019*
Group I vs Group III	Brilliant Everglow vs Heytec-N	0.000*	0.000*	0.000*	0.001*	0.006*	0.000*	0.000*	0.010*
Group II vs Group III	Beautifill II vs Heytec-N	0.923	0.022*	0.007*	1.000	1.000	0.000*	0.008*	1.000

*statistically significant.

Table 2
Correlation between Surface roughness and bacterial adherence.

		Surface roughness	Bacterial Adherence
Surface roughness	Pearson Correlation	1	.650**
	Sig. (2-tailed)		.000
	Sum of Squares and Cross-products	1.505	1028.047
	Covariance	.013	8.639
	N	120	120
Bacterial Adherence	Pearson Correlation	.650**	1
	Sig. (2-tailed)	.000	
	Sum of Squares and Cross-products	1028.047	1660095.867
	Covariance	8.639	13950.385
	N	120	120

**. Correlation is significant at the 0.01 level (2-tailed).

unable to remove or alter even the superficial filler layer of composite.[25]

Apart from surface roughness, bacterial adherence is dependent on other factors such as free surface energy, electrical properties and hydrophobicity of the substratum.[26] Also, the size and depth of depressions (surface topography) play an important role in biofilm formation. So, deeper and larger depressions which may be produced by loss of larger sized filler particles or prepolymerised filler lead to increased biofilm formation as compared to shallower and smaller depressions.[26] This can be explained as a reason for high bacterial adherence of Brilliant Everglow (large particle size) and Beautifil II (presence of s-PRG particles).

Beautifil II did not exhibit antibacterial activity in our study contrary to manufacturer's claims. This was because of degradation of F containing s-PRG particles after bleaching and plucking of these particles after ultrasonic scaling and powered toothbrushing resulting in deeper and larger depressions leading to increased bacterial adherence. In a study by **Yoshihara et al**[27] they suggested that Beautifil II samples containing sPRG fillers released different types of ions which contrary to results of the studies by **Miki et al**[28] and **Saku et al**[29] did not induce either contact, or regional antibacterial effects.

The limitations of the study were that the effect of saliva was not considered during bacterial adherence analysis. Saliva plays an important role as it provides flushing action and protective role in oral cavity, so further studies need to be done considering its role. The antibacterial activity of various s-PRG particles containing composites depend on critical pH in oral cavity for its activity. So, further clinical trials are needed to corroborate the results of our study.

7. Conclusion

Considering the limitations discussed, it can be inferred that Diode assisted Laser bleaching and ultrasonic scaling procedures may negatively affect the surface roughness and bacterial adherence of restorations irrespective of the composite used. Powered Toothbrushing did not affect the surface integrity of restoration. Therefore, it may be clinically advisable to do finishing and polishing of restorations after ultrasonic and laser procedures.

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