

Comparative evaluation of stains caused by using different mouth rinses on composite resin: An *in vitro* study

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Abstract

Background: Resin-based restorative materials have significantly evolved over the past decades and are widely used in dental practice to satisfy increasing esthetic demands. However, maintaining colour stability in the constantly changing oral environment remains a critical factor affecting their clinical longevity and continues to be a persistent challenge for these materials.

Material and methods: A total of 72 samples (n = 18) of microhybrid resin composite restorative material (Prime Dental Products, India) were prepared using a customized Teflon mold. Each specimen measured 10 mm in length, 10 mm in width, and 2 mm in thickness. The samples were randomly divided into four groups as follows: Group I: Control group (Artificial saliva) Group II: Oral B (non-alcohol-based mouthwash) Group III: Listerine (alcohol-based mouthwash) Group IV: Hexidine (chlorhexidine gluconate-based mouthwash) each group consisted of 18 samples. After the immersion procedure, values for each sample were recorded. The color differences were assessed using the CIE Lab* color space parameters: ΔL^* (white-black), Δa^* (red-green), and Δb^* (blue-yellow). The overall color change (ΔE^*ab) was calculated using the following formula.

Results: ΔE values differed significantly among all groups except Oral B and Hexidine, which showed no significant difference. Listerine showed the highest staining (mean $\Delta E = 5.208$), followed by Hexidine (3.14) and Oral B (3.03).

Conclusion: Within the limitations of this *in-vitro* study, the tested microhybrid composite exhibited color changes after immersion in all mouth rinses. The least color change was observed with Oral-B, whereas Listerine caused visually perceptible and clinically unacceptable discoloration. Hexidine demonstrated moderate color changes.

Keywords: Composite resin, Mouth rinse, Color stability, ΔE color change, *In vitro* study

Introduction

Patient awareness and expectations regarding dental esthetics have increased significantly in recent years. As a result, the success of restorative treatment depends not only on restoring function but also on achieving optimal esthetic outcomes^[1]. Composite resins are widely used in restorative dentistry to manage esthetic problems such as discoloration, diastemas, and minor dental malpositions^[2].

Despite continuous advancements since their introduction in the 1960s, composite restorations are still susceptible to discoloration in the oral environment^[3].

Resin-based restorative materials have evolved to meet aesthetic demands, but maintaining color stability in the oral environment remains a key challenge affecting their clinical longevity^[4].

Colour stability is association with the resin-matrix, dimension of its filler particles, "polymerization" degree and the colouring agents. Discolouration of composite resins can be caused by intrinsic or extrinsic factors^[5].

Intrinsic factors affecting composite color stability include the resin matrix, filler content, and stain absorption. Composites with lower filler and higher resin content absorb more water, leading to hydrolytic degradation and reduced color stability, while larger filler particles increase color permeability^[6].

Resins with a UDMA matrix show better color stability than Bis-GMA-based resins due to lower viscosity and water absorption. Larger filler particles increase surface roughness and staining, while higher polymerization efficiency reduces residual monomers and discoloration^[7].

External factors affecting color stability include staining from foods, beverages, smoking, and oral hygiene habits, as well as the type of colorant and exposure time. Common culprits include tea, coffee, wine, juices, nicotine, and mouth rinses^[7].

Mouthrinses can degrade resin composites through alcohol, detergents, emulsifiers, and acids. Essential oils like eucalyptol, thymol, menthol, and methyl salicylate may erode resin matrices, wear filler surfaces, and increase water sorption and solubility^[8, 9, 10].

Studies on mouthrinses and composite discoloration show variable results. Color changes are influenced not only by mouthrinses but also by food, beverages, bacteria, and the salivary pellicle. Different mouthrinses may also alter composite hardness and surface roughness, affecting color stability^[11, 12, 13].

Therefore, evaluating the effect of different mouth rinses on the color stability of composite resins is important for maintaining the esthetic longevity of restorations. Hence, the present study aims to compare and evaluate the staining effects of different mouth rinses on composite resin.

Materials and Methods

A total of 72 specimens (n = 18) of microhybrid resin composite (Prime Dental Products, India) were prepared using a customized Teflon mold with dimensions of 10 mm × 10 mm × 2 mm. The mold was positioned on a glass slab and filled with composite resin using a composite placement instrument. A second glass slab was placed over the mold and gently pressed for 30 seconds to obtain a uniform and smooth surface. Each specimen was light-cured using an LED curing unit (Monitex Industrial Co., Ltd., Taiwan) for 40 seconds twice. The thickness of the specimens was verified using a vernier caliper.

All specimens were stored in 20 ml of artificial saliva for 24 hours prior to baseline color measurement using a spectrophotometer (VITA Zahnfabrik, Germany). The samples were randomly divided into four groups (n = 18): Group I – Artificial saliva (control), Group II – Oral B (non-alcohol mouthwash), Group III – Listerine (alcohol-based mouthwash), and Group IV – Hexidine (chlorhexidine mouthwash).

Specimens were immersed in 20 ml of their respective solutions for 21 minutes, simulating daily one-minute mouthrinse use. After each immersion, samples were transferred to artificial saliva for a 12-hour interval before reimmersion in fresh solution. This cycle was repeated eight times over four days to simulate six months of mouthrinse exposure. Final color measurements were recorded using a spectrophotometer.

Color changes were evaluated using the CIE Lab* color system, and overall color change (ΔEab) was calculated using the formula:

$$\Delta E_{ab} = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]}$$

Statistical analysis

Statistical analysis performed with IBM SPSS 20 software. ANOVA test followed by Tukey’s post hoc test with significance at p ≤ 0.05.

Results

Descriptive and inferential statistical analyses were performed. Data were entered in Microsoft Excel 2010, and

continuous variables were expressed as Mean ± SD. A p-value ≤ 0.05 was considered statistically significant.

Table 3, Group I (Artificial saliva, control; n=18) showed ΔE values ranging from 0.95–2.15 with a mean of 1.36 ± 0.35. Group II (Oral B; n=18) showed ΔE values of 2.283–3.703 with a mean of 3.025 ± 0.41. Group III (Listerine; n=18) showed ΔE values ranging from 4.295–6.309 with a mean of 5.208 ± 0.58. Group IV (Hexidine; n=18) showed ΔE values of 2.975–3.344 with a mean of 3.140 ± 0.10.

Table 4 shows the results of the ANOVA analysis for comparison between and within the groups. The sum of squares for between-group variation was 134.104 with 3 degrees of freedom, while the within-group variation had a sum of squares of 10.991 with 68 degrees of freedom. The obtained p-value was 0.000 (<0.05), indicating that the ΔE values among the groups were statistically significant.

Table 5, Graphs 2, 3, 4, and 5 present the results of the post hoc Tukey analysis, which was performed due to the statistically significant differences observed in the ANOVA test.

The mean difference between Group I (Artificial saliva/control) and Group II (Oral B) was -1.663, which was statistically significant (p < 0.001). Similarly, the mean difference between Group I and Group III (Listerine) was -3.847 (p < 0.001), and between Group I and Group IV (Hexidine) was -1.780 (p < 0.001), indicating statistically significant differences.

The comparison between Group II (Oral B) and Group III (Listerine) also showed a statistically significant mean difference of -2.183 (p < 0.001).

However, the difference between Group II (Oral B) and Group IV (Hexidine) was not statistically significant, with a mean difference of -0.116 (p = 0.822).

The mean difference between Group III (Listerine) and Group IV (Hexidine) was 2.067, which was statistically significant (p < 0.001).

Overall, ΔE values differed significantly among all groups except Oral B and Hexidine, which showed no significant difference. Listerine showed the highest staining (mean ΔE = 5.208), followed by Hexidine (3.14) and Oral B (3.03).

Thus, the null hypothesis was rejected, as all tested mouthrinses caused significant color change in composite resin.

Table 3: Descriptive statistics for ΔE for all groups

Groups	Count	Mean	Std. Deviation	Variance	Minimum	Maximum
Group I (Control Group)	18	1.36	0.34	0.12	0.95	2.15
Group II (Oral B)	18	3.03	0.41	0.17	2.28	3.70
Group III (Listerine)	18	3.42	0.35	0.12	4.30	6.31
Group IV (Hexidine)	18	5.22	0.66	0.44	2.97	3.34

Table 4: Comparison of ΔE between the groups & within groups (ANNOVA)

Source of Variation	Sum of Square	Degree of Freedom	Mean Square	F	p-value
Between Groups	134.104	3	44.701	276.572	0.000
Within Groups	10.991	68	0.162		
Total	145.095	71			

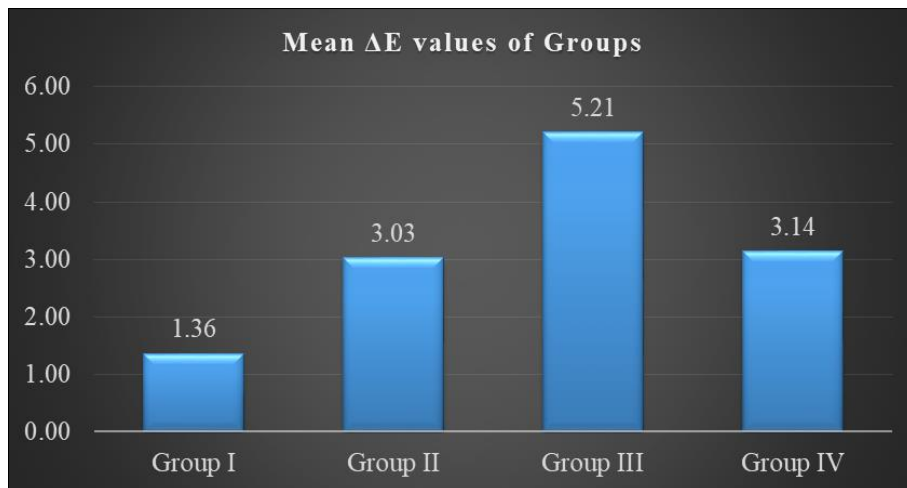
Table 5: Pairwise Comparison between Groups Post Hoc Tukey Test

Group (A)	Group (B)	Mean Difference (A-B)	Std. Error	p Value	Inference
Group I	Group II	-1.664	0.134	0.000	Significant
	Group III	-3.848	0.134	0.000	Significant
	Group IV	-1.780	0.134	0.000	Significant
Group II	Group I	1.664	0.134	0.000	Significant
	Group III	-2.184	0.134	0.000	Significant

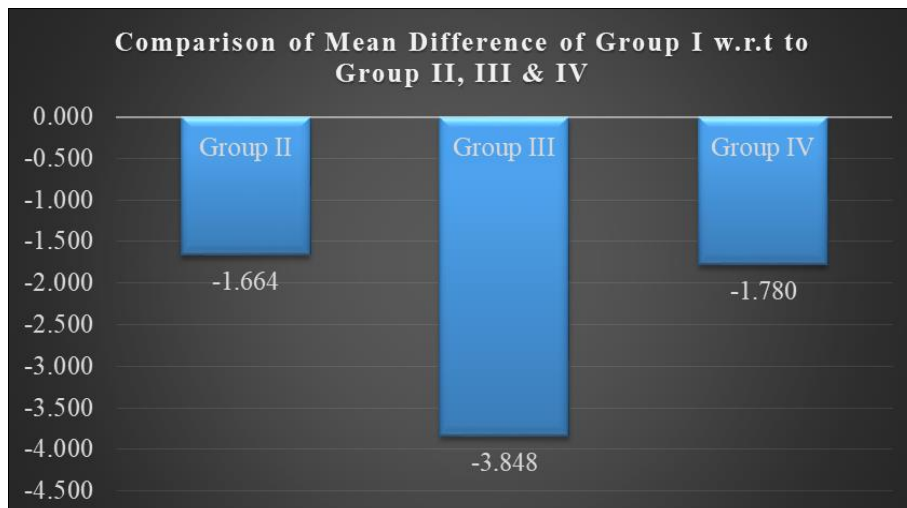
	Group IV	-0.116	0.134	0.822	Non-Significant
Group III	Group I	3.848	0.134	0.000	Significant
	Group II	2.184	0.134	0.000	Significant
	Group IV	2.068	0.134	0.000	Significant
Group IV	Group I	1.780	0.134	0.000	Significant
	Group II	0.116	0.134	0.822	Non-Significant
	Group III	-2.068	0.134	0.000	Significant

Table 6: ΔE calculated for each group

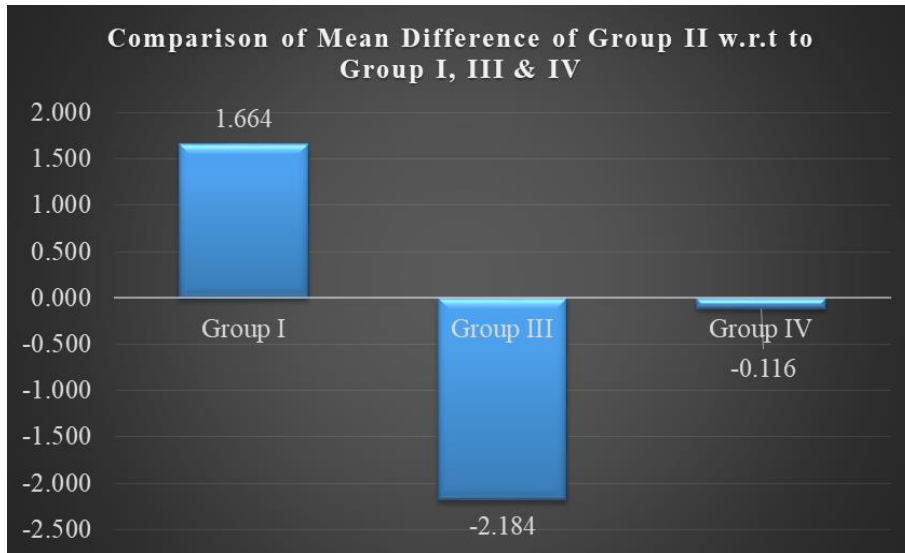
Sr. No.	Group I (Control Group)	Group II (Oral B)	Group III (Listerine)	Group IV (Hexidine)
1	1.32	3.10	6.31	3.34
2	1.95	3.22	6.16	3.01
3	1.69	3.70	5.54	3.23
4	1.66	3.28	4.87	3.11
5	1.45	2.69	5.91	3.04
6	0.95	2.61	5.41	3.13
7	1.02	2.70	5.69	3.17
8	1.08	2.28	5.45	3.11
9	1.05	2.83	5.76	3.28
10	1.44	3.13	4.68	3.05
11	1.64	3.40	4.81	3.06
12	1.14	2.94	5.04	3.20
13	0.97	3.56	4.49	3.18
14	1.36	3.60	5.02	3.17
15	1.12	2.34	4.66	3.28
16	1.36	3.09	4.87	3.06
17	2.15	3.20	4.79	3.15
18	1.15	2.78	4.30	2.97



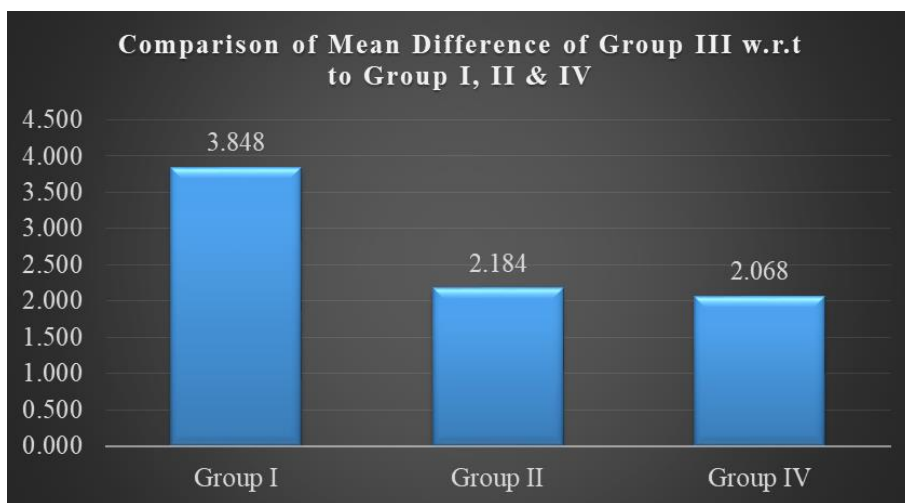
Graph 1: Mean ΔE Values of all Groups



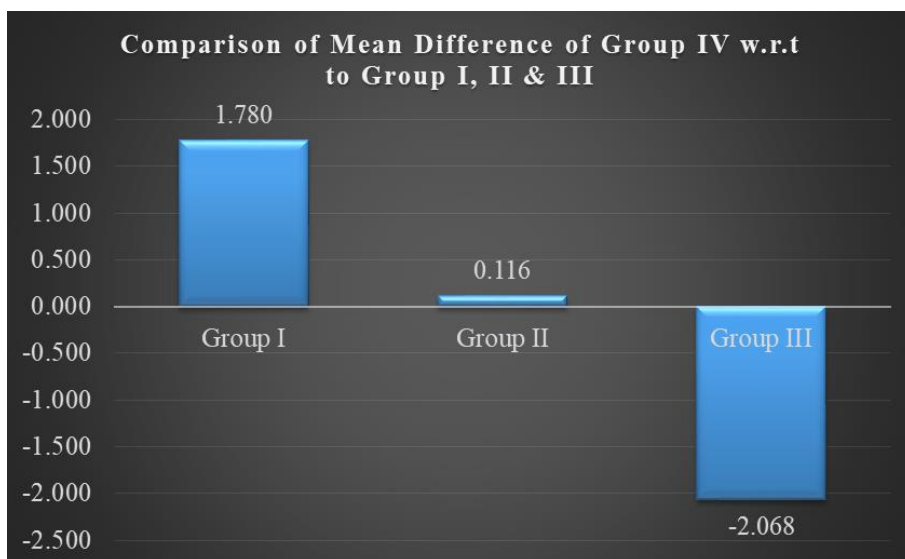
Graph 2: Comparison of Mean Difference of Group I w.r.t to Group II, III & IV



Graph 3: Comparison of Mean Difference of Group II w.r.t to Group I, III & IV



Graph 4: Comparison of Mean Difference of Group III w.r.t to Group I, II & IV



Graph 5: Comparison of Mean Difference of Group IV w.r.t to Group I, II & III

Discussion

Significant color changes were observed in all composite groups immersed in artificial saliva and mouth rinses. Among the tested solutions, Oral-B (alcohol-free) caused significantly less color change compared with Listerine

(alcohol-based) and Hexidine (chlorhexidine-based) mouth rinses.

In artificial saliva, water sorption within the polymer matrix can lead to expansion of polymer chains, leaching of unreacted monomers, and surface roughening, which

increases susceptibility to staining. In this study, ΔE in the control group (1.36) was clinically non-perceptible, consistent with previous reports [14].

Oral-B, despite being alcohol-free, produced a moderate ΔE (3.03). Ingredients such as solvents, fluoride, and acids may soften the resin matrix, dislodging filler particles and creating surface irregularities. These changes facilitate stain penetration, although the overall color change remained below the clinically perceptible threshold [15].

Listerine caused the highest color change ($\Delta E = 5.21$). Its low pH and high alcohol content accelerate hydrolytic degradation of ester bonds in dimethacrylate monomers (Bis-GMA, UDMA, TEGDMA). Hydrolysis weakens the polymer network, increases surface roughness, and enhances susceptibility to staining. These findings align with prior studies showing alcohol-containing, acidic mouth rinses significantly impact composite color stability [16].

Hexidine produced minimal but measurable discoloration ($\Delta E = 3.14$). Chlorhexidine is known for its substantivity and antimicrobial efficacy, but extrinsic staining usually requires dietary chromogens, which were absent in this *in-vitro* study. Minor intrinsic staining may result from water sorption due to hydrophilic monomers like TEGDMA. Hence, color changes with Hexidine were not clinically perceptible [17].

Comparisons between groups revealed that Oral-B vs. Listerine and Listerine vs. Hexidine showed statistically significant differences, reflecting Listerine's higher staining potential. In contrast, Oral-B vs. Hexidine was not significant, indicating similar minimal effects on composite color under the tested conditions. These results highlight that mouth rinse composition—particularly alcohol content and pH—plays a crucial role in composite discoloration, while hydrophilic monomers contribute to intrinsic changes. Overall, all tested mouth rinses produced measurable color changes, with Listerine showing the highest staining, followed by Hexidine, and Oral-B the lowest. Artificial saliva caused only minor, clinically non-perceptible changes. These findings underscore the importance of selecting appropriate oral hygiene products, especially for patients with resin-based restorations, to maintain long-term aesthetic outcomes.

Conclusion

Under the conditions of this *in-vitro* study, the tested microhybrid composite showed color changes after immersion in all mouth rinses. Oral-B caused the least change, while Listerine produced visually perceptible and clinically unacceptable discoloration, and Hexidine caused moderate changes.

These results highlight the impact of mouth rinse composition on the aesthetic stability of resin composites. Clinicians should inform patients—especially those using Listerine—about its higher potential for staining. Long-term *in vivo* studies are needed to further evaluate composite color stability.

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