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Clear aligner surface roughness and color stability following mouthwash exposure - An *in vitro* study

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Abstract:
Patients undergoing orthodontic treatment with clear aligners frequently use mouthwashes for oral hygiene, yet the chemical agents in these rinses may compromise the surface integrity and esthetics of aligner materials, potentially affecting treatment outcomes. Therefore, it is of interest to evaluate the effects of different mouthwashes on the surface roughness and color stability of clear aligner materials. Forty thermoplastic aligner samples (PET-G) were divided into four groups: control, chlorhexidine, alcohol-based and herbal mouthwash. All mouthwashes increased surface roughness and caused color changes, with the alcohol-based mouthwash producing the greatest degradation, while herbal mouthwash showed the least adverse effect. Thus, data shows that herbal mouthwash offers a clinically safer alternative for patients wearing polyethylene terephthalate glycol (PET-G) clear aligners, supporting evidence-based recommendations for oral hygiene protocols during aligner therapy.

Keywords: Clear aligners, surface roughness, color stability, mouthwashes, thermoplastic materials

Background:

Clear aligner therapy has gained significant popularity in contemporary orthodontics due to its superior esthetics, patient comfort and removability compared to conventional fixed appliances **[1]**. The introduction of thermoplastic materials such as polyethylene terephthalate glycol (PET-G) and polyurethane has enabled the fabrication of transparent aligners capable of delivering controlled orthodontic forces **[2].** Despite these advantages, aligners are continuously exposed to the dynamic oral environment, which includes fluctuations in temperature, salivary enzymes and various chemical agents that may alter their physical and optical properties over time **[3]**. Surface roughness is a critical factor influencing the clinical performance of orthodontic materials. Increased surface irregularities facilitate bacterial adhesion and biofilm accumulation, which may compromise oral hygiene and increase the risk of enamel demineralization and periodontal inflammation during treatment **[4]**. Thermoplastic materials used in clear aligners are particularly susceptible to surface degradation when exposed to oral conditions and chemical agents, leading to increased roughness and reduced material integrity **[5].** These changes may not only affect the mechanical efficiency of aligners but also contribute to microbial colonization. Color stability is another essential parameter, as the esthetic success of aligner therapy relies on the transparency of the material. Discoloration of aligners can negatively impact patient satisfaction and compliance. Previous studies have showed that clear aligner materials are prone to staining when exposed to commonly consumed beverages and oral solutions **[6]**. The degree of discoloration depends on factors such as material composition, duration of exposure and the chemical nature of the staining agents **[7]**. Spectrophotometric analysis using the CIELAB color system is widely accepted as a reliable method to quantify these changes **[8]**. Mouthwashes are commonly prescribed as adjuncts for maintaining oral hygiene, particularly in orthodontic patients who are at increased risk of plaque accumulation. Chlorhexidine gluconate remains the gold standard antimicrobial mouthwash due to its proven efficacy; however, it is also associated with adverse effects such as staining of teeth and restorative materials **[9].** Alcohol-containing mouthwashes may alter the structural integrity of polymer-based materials by increasing surface porosity and promoting degradation **[10]**. In contrast, herbal mouthwashes have gained attention due to their biocompatibility and minimal side effects, although their effects on orthodontic materials remain inadequately explored **[11]**. Recent studies have reported that exposure of clear aligners to various chemical agents, including cleansing solutions and oral rinses; can result in significant changes in surface roughness, translucency and color stability **[12]**. However, there is limited comparative evidence evaluating the effects of different categories of mouthwashes on clear aligner materials under simulated clinical conditions **[13]**. Therefore, it is of interest to evaluate and compare the effects of different mouthwashes on the surface roughness and color stability of clear aligner materials.

Materials and Methods:

The present study was designed as an *in vitro* experimental study to evaluate the effect of different mouthwashes on the surface roughness and color stability of clear aligner materials under simulated clinical conditions. The sample size was calculated based on previous in vitro studies evaluating surface and optical properties of orthodontic thermoplastic materials, with a confidence level of 95% and a statistical power of 80%. A total of 40 samples were included and equally distributed into four groups (n = 10 per group). Commercially available thermoplastic clear aligner sheets (PET-G based material, thickness: 1 mm) were used for sample preparation. The material was selected due to its widespread clinical use in clear aligner therapy. The inclusion criteria for the study involved uniform thermoplastic aligner sheets with consistent thickness (1 mm), free from visible defects, scratches, or porosities. The specimens were standardized to dimensions of 10 mm × 10 mm × 1 mm and fabricated from the same batch of material. Exclusion criteria included samples with surface irregularities, uneven thickness and distortion during cutting, contamination, or pre-existing discoloration or deformation. For sample preparation, the thermoplastic sheets were thermoformed and sectioned into standardized specimens using a precision cutting device. These specimens were polished gently to remove sharp edges without altering their surface characteristics. They were then ultrasonically cleaned in distilled water for 10 minutes to eliminate any surface contaminants, followed by air drying and storage in a sterile environment until testing. The prepared samples were randomly divided into four groups, with 10 samples in each group: Group I (Control) immersed in distilled water, Group II immersed in 0.2% Chlorhexidine mouthwash, Group III immersed in alcohol-based mouthwash and Group IV immersed in herbal mouthwash. The immersion protocol involved immersing each sample in 10 mL of the respective solution for 15 minutes daily. After each immersion, the samples were rinsed with distilled water and stored in artificial saliva at 37°C between immersion cycles. This procedure was repeated for 14 consecutive days, simulating approximately two weeks of clinical use. Artificial saliva was refreshed daily to ensure consistency. Surface roughness (Ra, μm) was measured using a contact profilometer at baseline and after 14 days, with three readings taken at different locations on each specimen. The mean value was calculated for each sample using standardized parameters: a cut-off value of 0.8 mm and a tracing length of 4 mm. Color stability was assessed using a spectrophotometer based on the CIELAB color system, recording the parameters L* (lightness), a* (red-green axis) and b* (yellow-blue axis) at both baseline and after 14 days. The color difference (ΔE) was calculated using the formula $\Delta E = \sqrt{[(L1 - L0)^2 + (a1 - a0)^2 + (b1 - b0)^2]}$, where L_0, a_0, b_0 are baseline values and L_1, a_1, b_1 are post-immersion values. The primary outcome measures included surface roughness (Ra) and color change (ΔE), with the secondary outcome comparing the different mouthwash groups. Statistical analysis was conducted using SPSS software (Version 25.0, IBM Corp. and USA). Descriptive statistics were calculated for all data and a paired t-test was used to compare baseline and post-immersion values within each group. One-way ANOVA was applied for intergroup comparisons and a post hoc Tukey test was used for multiple comparisons. A significance level of $p < 0.05$ was set for all analyses.

Results:

All samples completed the study without any surface defects or fractures. The data obtained for surface roughness (Ra) and color change **(ΔE)** were analyzed and are presented below. Table 1 shows the comparison of mean surface roughness (Ra) values at baseline and after 14 days. At baseline, all groups showed nearly similar surface roughness values, indicating uniformity of samples prior to immersion. After 14 days, an increase in surface roughness was observed in all groups. The control group exhibited only a minimal increase, whereas the chlorhexidine group showed a moderate rise in roughness values. The alcohol-based mouthwash group showed the highest increase in surface roughness, indicating maximum surface degradation. In contrast, the herbal mouthwash group showed a comparatively smaller increase, suggesting minimal alteration of the aligner surface. Table 2 presents the intergroup comparison of surface roughness values at day 14 using ANOVA and Tukey post hoc analysis. Statistically significant differences were observed among all groups ($p < 0.05$). The alcohol-based mouthwash group showed significantly higher surface roughness compared to all other groups. The chlorhexidine group also demonstrated significantly higher roughness compared to the control and herbal groups. Although the herbal mouthwash group showed a statistically significant increase when compared to the control group, the magnitude of change was considerably lower than that observed in the other experimental groups. Table 3 illustrates the mean color change (ΔE) values of all groups after 14 days. The control group exhibited minimal color change, which was not clinically perceptible. The chlorhexidine group demonstrated the highest ΔE values, exceeding the clinically acceptable threshold, indicating significant discoloration. The alcohol-based mouthwash group showed moderate color change, which was clinically noticeable. In contrast, the herbal mouthwash group exhibited relatively low ΔE values, remaining within clinically acceptable limits. Table 4 shows the intergroup comparison of color change values using ANOVA and Tukey post-hoc analysis. A statistically significant difference was observed among all groups ($p < 0.05$). The chlorhexidine group showed significantly higher discoloration compared to all other groups. The alcohol-based group also showed significantly greater color change than the control and herbal groups. Although the herbal mouthwash group showed a statistically significant increase compared to the control, the extent of discoloration remained within clinically acceptable limits, indicating better color stability compared to other experimental groups.

Table 1: Comparison of surface roughness (Ra, μm) at baseline and after 14 days

Group	Baseline (Mean ± SD)	Day 14 (Mean ± SD)	Mean Difference	Paired t-test (p-value)
Control (Distilled Water)	0.214 ± 0.011	0.221 ± 0.013	+0.007	0.084 (NS)
Chlorhexidine	0.215 ± 0.012	0.296 ± 0.016	+0.081	<0.001*
Alcohol-based Mouthwash	0.213 ± 0.010	0.358 ± 0.019	+0.145	<0.001*

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Herbal Mouthwash	0.214 ± 0.011	0.249 ± 0.014	+0.035	0.003*

Statistically significant ($p < 0.05$)

Table 2: Intergroup comparison of surface roughness (Ra) after 14 days

Parameter	F-value	p-value
Surface Roughness (Ra)	142.61	<0.001*
One-way ANOVA		
Comparison	**Mean Difference**	**p-value**
Control vs Chlorhexidine	-0.075	<0.001*
Control vs Alcohol	-0.137	<0.001*
Control vs Herbal	-0.028	0.021*
Chlorhexidine vs Alcohol	-0.062	<0.001*
Chlorhexidine vs Herbal	0.047	<0.001*
Alcohol vs Herbal	0.109	<0.001*

Tukey Post Hoc Analysis

Table 3: Comparison of color change (ΔE) After 14 Days

Group	ΔE (Mean ± SD)
Control (Distilled Water)	1.12 ± 0.28
Chlorhexidine	5.48 ± 0.56
Alcohol-based Mouthwash	3.89 ± 0.47
Herbal Mouthwash	2.05 ± 0.35

Table 4: Intergroup comparison of color change (ΔE)

Comparison	Mean Difference	p-value
Control vs Chlorhexidine	-4.36	<0.001*
Control vs Alcohol	-2.77	<0.001*
Control vs Herbal	-0.93	0.014*
Chlorhexidine vs Alcohol	1.59	<0.001*
Chlorhexidine vs Herbal	3.43	<0.001*
Alcohol vs Herbal	1.84	<0.001*

Tukey Post Hoc Analysis

Discussion:

The present *in vitro* study evaluated the effect of different mouthwashes on the surface roughness and color stability of clear aligner materials, simulating short-term clinical exposure. The findings showed that both parameters were significantly influenced by the type of mouthwash used, with alcohol-based and chlorhexidine mouthwashes producing greater alterations compared to herbal formulations. These results highlight the importance of chemical exposure in determining the physical and esthetic behavior of thermoplastic aligner materials. Surface roughness is a critical parameter influencing the biological performance of orthodontic materials. Increased surface irregularities facilitate bacterial adhesion and plaque accumulation, thereby increasing the risk of enamel demineralization and periodontal complications. Alkattan (2025) *et al.* **[14]** established that even minimal increases in surface roughness can significantly enhance microbial colonization on oral materials. In the present study, all experimental groups showed an increase in surface roughness, with the highest values observed in the alcohol-based mouthwash group. The effect of chemical agents on orthodontic materials has been increasingly investigated in recent literature. Gkinosati *et al.* (2025) **[15]** conducted a systematic review and reported that mouthwashes can alter the morphology, structure and mechanical properties of orthodontic materials. This finding is consistent with the present results, where chemical exposure resulted in measurable surface degradation. Aşık *et al.* **[16]** further demonstrated that different cleaning protocols significantly influence the optical and surface properties of clear aligners, indicating that routine oral hygiene practices may impact material integrity. Chlorhexidine mouthwash also resulted in a significant increase in surface roughness. Zanatta *et al.* (2007) **[17]** reported that chlorhexidine can interact with oral surfaces and lead to chemical alterations, including deposition of substances that may affect material characteristics. This supports the present findings, where chlorhexidine exposure caused noticeable surface changes, although less pronounced than alcohol-based solutions. The relatively moderate effect of chlorhexidine compared to alcohol-based mouthwash may be attributed to differences in their chemical composition and interaction with polymer matrices. The influence of external factors on aligner usage and performance has also been highlighted in the literature. Similarly, Eliades and Eliades (2024) **[18]** reported that aligner materials undergo measurable wear and physical changes during usage, which may alter their surface topography. These findings support the present study, suggesting that both mechanical and chemical factors contribute to surface degradation of aligners. The clinical implications of these changes are further supported by studies evaluating periodontal outcomes during aligner therapy. Jiang *et al.* (2018) **[19]** reported that changes in aligner surfaces may influence plaque accumulation and periodontal health in patients undergoing orthodontic treatment. Crego-Ruiz and Jorba-García (2023) **[20]** also showed that aligner therapy can affect periodontal parameters, emphasizing the importance of maintaining material integrity to prevent adverse oral health outcomes. These findings underline the clinical relevance of surface roughness changes observed in the present study. From a materials science perspective, the degradation of thermoplastic polymers is influenced by their interaction with chemical agents. Aldawood (2026) **[21]** reported that dental polymers undergo physicochemical changes when exposed to external agents, affecting their mechanical and surface properties. Similarly, Bociong *et al.* (2017) **[22]** showed that water sorption and chemical interactions can alter the structural integrity of polymeric materials. These mechanisms may explain the increased surface roughness observed in the alcohol-based and chlorhexidine groups in the present study. In contrast, the herbal mouthwash group showed comparatively lower surface roughness values. Prabu *et al.* (2006) **[23]** identified plant-derived compounds such as guaijaverin as effective antiplaque agents with reduced chemical aggressiveness. These findings support the present results, suggesting that herbal formulations may be less detrimental to aligner materials and may serve as safer alternatives for long-term use. Color stability is another critical factor influencing the esthetic success of clear aligner therapy. In the present study, chlorhexidine mouthwash produced the highest color change (ΔE), followed by alcohol-based mouthwash, while the herbal group showed minimal discoloration. Venkatasubramanian *et al.* (2022) **[24]** showed that exposure to food substances can significantly affect the color stability of aligner materials. The staining potential of chlorhexidine has been extensively documented. Zanatta *et al.*

(2007) **[17]** reported that chlorhexidine causes discoloration through interaction with chromogenic compounds and formation of pigmented deposits. This mechanism likely contributed to the higher ΔE values observed in the present study. Joiner also emphasized that chemical interactions between materials and external agents play a crucial role in extrinsic staining. Alcohol-based mouthwash also resulted in considerable discoloration, which may be attributed to increased surface porosity facilitating stain absorption. Olteanu *et al.* (2024) **[25]** showed that exposure to staining beverages leads to significant color changes in clear aligners, highlighting the susceptibility of these materials to external agents. These findings are consistent with the present results, where chemical exposure enhanced discoloration. Recent studies have further emphasized the importance of cleaning protocols in maintaining aligner esthetics. Silva *et al.* **[26]** reported that different cleaning solutions significantly affect both the microbiological and colorimetric properties of clear aligners. This highlights the role of routine oral hygiene practices in determining the long-term appearance of aligner materials. From a clinical perspective, increased surface roughness may promote plaque accumulation, while discoloration can negatively impact patient satisfaction and compliance. Therefore, the selection of mouthwash during aligner therapy is of considerable importance. Based on the findings of the present study, alcohol-based and chlorhexidine mouthwashes should be used with caution, whereas herbal mouthwashes may be considered safer alternatives. However, certain limitations must be acknowledged. Being an *in vitro* study, it does not fully replicate intraoral conditions such as salivary flow, masticatory forces and microbial activity. Additionally, the study evaluated short-term exposure and long-term effects require further investigation. Future studies incorporating *in vivo* conditions and different aligner materials are recommended to validate these findings and provide more comprehensive clinical guidance.

Conclusion:

Alcohol-based mouthwash produced the greatest increase in aligner surface roughness. Chlorhexidine caused the highest discoloration, while herbal mouthwash showed the least changes. Selecting an appropriate mouthwash may improve aligner durability and esthetic outcomes.

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