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Comparative Evaluation of Patient-Performed Cleaning Methods on the Integrity of Clear Orthodontic Aligners: A Randomized Clinical Trial

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Abstract:

- ***Introduction:***

Clear aligner therapy offers an aesthetic and removable alternative to conventional orthodontic appliances. However, aligners are continuously exposed to the oral environment and may undergo changes in surface characteristics, colour and biological contamination during clinical use. Appropriate cleaning is therefore important for maintaining aligner integrity and hygiene. The present study evaluated the effects of commonly used patient-performed cleaning methods on clear orthodontic aligners.

- ***Aim:***

To compare the effects of five cleaning methods on the surface roughness, colour change and ATP bioluminescence of clear orthodontic aligners following 14 days of intraoral use.

- ***Materials and Methods:***

An in-vivo comparative study was conducted among 60 participants, randomly allocated into five groups of 12 each: soap solution, Orodox cleansing tablets, Toothsi cleaning foam, water (control), and baking soda paste. Each aligner was used for 14 days and cleaned daily according to the assigned protocol. Surface roughness was evaluated using a surface profilometer and expressed as Ra (µm). Colour change was assessed using a spectrophotometer and expressed as ΔE. Biological contamination was assessed using an ATP bioluminometer and expressed as relative light units (RLU). Data were analysed using IBM SPSS Statistics. One-way ANOVA followed by Tukey's HSD post-hoc test was used for surface roughness and colour change. Since the homogeneity of variance assumption was violated for ATP, Welch's ANOVA followed by Games–Howell post-hoc analysis was performed. A p-value <0.05 was considered statistically significant.

- ***Results:***

Statistically significant differences were observed among the five cleaning groups for all three parameters ($p < 0.001$). The lowest mean surface roughness was observed with Orodox tablets (0.220 ± 0.032 µm), while the highest was observed with water (0.418 ± 0.065 µm). Mean colour change was lowest in the Orodox group (1.18 ± 0.293) and highest in the water group (3.05 ± 0.458). ATP values were also lowest for Orodox (82.00 ± 20.122 RLU) and highest for water (415.08 ± 60.614 RLU). One-way ANOVA demonstrated significant differences for surface roughness ($F = 31.633$, $p < 0.001$) and colour change ($F = 48.913$, $p < 0.001$). Welch's ANOVA demonstrated a significant difference in ATP values ($F = 92.401$, $p < 0.001$). Games–Howell analysis showed that Orodox and Toothsi did not differ significantly for ATP ($p = 0.109$).

- ***Conclusion:***

Within the limitations of the present study, Orodox cleansing tablets demonstrated the most favourable overall performance, with the lowest surface roughness, colour change and ATP values following 14 days of aligner use. Toothsi foam demonstrated comparable results, whereas water alone showed the least favourable profile across the evaluated

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parameters. Further studies with larger samples and longer observation periods are warranted to determine the long-term effects of different aligner cleaning methods.

Keywords: *Clear Aligners; Aligner Cleaning; Surface Roughness; Colour Stability; ATP Bioluminescence; Orthodontic Aligners; Orodox; Toothsi; Baking Soda; Oral Hygiene.*

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I. INTRODUCTION

Clear aligner therapy has become an increasingly popular orthodontic treatment modality because of its aesthetic appearance, removability and patient acceptance. Clear aligners are generally worn for approximately 1–2 weeks before replacement, during which time they remain continuously exposed to the oral environment. Saliva, food debris, microorganisms and dietary substances can accumulate on the aligner surfaces and may contribute to plaque formation, staining and changes in the physical properties of the thermoplastic material. [1,2,3]

Maintenance of aligner hygiene is therefore important not only for oral health but also for preserving the transparency and surface characteristics of the appliance. Inadequate cleaning may result in microbial accumulation, surface deposits and discoloration, whereas unsuitable or aggressive cleaning procedures may adversely affect the properties of the aligner material. The study by Athar Alweneen and Nasser Alqahtani similarly emphasizes that proper cleaning is important for preserving both hygiene and optical properties of clear aligners. [4,5,6,7]

Several cleaning methods are currently used by patients, including commercially available cleansing products, water and home-based cleaning remedies. However, the effectiveness and material compatibility of these approaches may differ. The Alweneen and Alqahtani study compared tooth brushing with whitening toothpaste, vinegar, Fittydent Super Cleansing Tablets, Invisalign cleaning crystals and water, and evaluated physical, mechanical, chemical and optical properties of clear aligners. [13]

Surface roughness is an important characteristic of aligner materials because changes in surface texture may influence plaque accumulation and staining. Clinical use has been associated with increased surface roughness and alterations in material properties. [6,8,11]

Colour stability is another important consideration because transparency is one of the primary advantages of clear aligner therapy. Discoloration may compromise the aesthetic appearance of the appliance and potentially affect patient satisfaction. [13]

Microbial contamination is also clinically relevant because aligners are worn in close contact with teeth and saliva for prolonged periods. ATP bioluminescence provides a rapid quantitative assessment of biological contamination and was therefore incorporated into the present study. [7]

The present study was undertaken to compare five patient-performed cleaning methods—soap solution, Orodox cleansing tablets, Toothsi cleaning foam, water and baking soda paste—and to evaluate their effects on surface roughness, colour stability and biological contamination of clear orthodontic aligners under in-vo conditions. [13]

➢ *Aim and Objectives*

To evaluate and compare the effect of commonly used patient-performed cleaning methods on the integrity of clear orthodontic aligners under in-vivo conditions.

The objectives of the present study were to evaluate the effect of different patient-used cleaning methods on the surface integrity of clear orthodontic aligners following intraoral use, assess their colour stability following routine cleaning with commercial cleansers, water and a home-based cleaning method, and compare the effectiveness of the different cleaning methods in maintaining the aesthetic and surface characteristics of clear aligners.

II. MATERIALS AND METHODS

➢ *Study Design*

The present study was an in-vivo comparative study conducted among patients undergoing orthodontic clear aligner treatment. The study was conducted in the Department of Orthodontics and Dentofacial Orthopaedics, KVG Dental College and Hospital, Sullia, with a total sample of 60 patients and a study duration of 3 months.

➢ *Sample Size*

The sample size was calculated using:

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

Where:

- SD = standard deviation
- $Z_{1-\alpha/2}$ = 1.96 at 95% confidence interval
- $Z\beta$ = 0.84 at 80% power
- d = mean difference

The calculated sample size was approximately 10.8, rounded to 11. Considering a 10% sample loss, the final sample size was increased to 12 participants per group, resulting in a total sample size of 60 participants.

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➢ *Study Groups*

The 60 participants were randomly allocated into five groups according to the cleaning method:

- Group 1 ; soap solution with mild surfectants
- Group 2 ; Orodox® cleansing tablets
- Group 3 ; Toothsi® cleaning foam
- Group 4 ; water (as control)
- Group 5 ; baking soda paste

➢ *Selection Criteria*

Patients undergoing clear aligner orthodontic treatment, aged 18 years and above, with good general and oral health and willing to comply with study instructions and provide informed consent were included.

Patients with poor oral hygiene or active periodontal disease, systemic conditions affecting oral health, use of unassigned or additional aligner cleaning agents, smoking or tobacco-use habits, or known allergy to any of the cleaning agents were excluded.

➢ *Materials Used*

The materials and equipment included clear orthodontic aligners fabricated from thermoplastic material, soap solution with mild surfactants, Orodox cleansing tablets, Toothsi aligner cleaning foam, water, baking soda paste, distilled water, sterile containers, a spectrophotometer, surface profilometer, ATP bioluminometer and personal protective equipment. [4,5]

➢ *Cleaning Protocol*

All participants were instructed to wear their aligners according to routine clinical protocol (14 days) and to clean them once daily using only the assigned cleaning method. Participants were instructed not to use additional or alternative cleaning agents during the study period.

- Group 1 (Soap Solution) ; One milliliter of soap solution was diluted in 240 mL of water. The aligner was completely submerged in the solution for 2 minutes and subsequently rinsed under running water for 30 seconds.
- Group 2 (Orodox Tablets) ; One Orodox cleansing tablet was dissolved in 150 mL of water. The aligner was soaked for 10–15 minutes and then thoroughly rinsed under running water for 30 seconds.
- Group 3 (Toothsi Foam) ; Toothsi cleaning foam was applied to both the inner and outer surfaces of the aligner and left for 30 seconds, followed by thorough rinsing under running water.
- Group 4 (Water Control) ; The aligner was cleaned under running water using the finger on both the inner and outer surfaces.
- Group 5 (Baking Soda Paste) ; The paste was prepared by mixing 2 parts baking soda with 1 part water, specifically 2 tablespoons of baking soda with 1 tablespoon of water. The paste was applied to the aligner for 1 minute and then rinsed under running water.

➢ *Collection of Aligners*

At the end of the 14-day usage period, the aligners were collected, rinsed with distilled water, air-dried and subjected to laboratory evaluation. The approved methodology specifies collection followed by distilled-water rinsing and air drying before evaluation.

➢ *Outcome Measures*

Colour change was evaluated using a spectrophotometer and expressed as ΔE values. [13]

Surface roughness was evaluated using a surface profilometer and expressed as Ra in µm. [6,8,11]

Microbial contamination was evaluated using ATP bioluminescence, with results expressed as relative light units (RLU). [7]

➢ *Statistical Analysis*

Statistical analysis was performed using IBM SPSS Statistics. Descriptive statistics were calculated and expressed as mean ± standard deviation (SD) for all continuous variables. The normality of the data was assessed using the Shapiro–Wilk test, while homogeneity of variance was assessed using Levene's test.

Intergroup comparisons for surface roughness and colour change were performed using one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) post-hoc test for pairwise comparisons.

For ATP bioluminescence, Levene's test indicated violation of the homogeneity of variance assumption. Therefore, Welch's ANOVA was used for overall group comparison, followed by Games–Howell post-hoc analysis for pairwise comparisons.

A p-value <0.05 was considered statistically significant.

III. RESULTS

A total of 60 participants were included, with 12 participants in each of the five study groups. Following 14 days of aligner use, the aligners were assessed for surface roughness, colour change and ATP bioluminescence.

➢ *Descriptive Statistics*

The Orodox Tablet group demonstrated the lowest mean values for all three parameters, whereas the Water Control group demonstrated the highest mean values.

Table 1 Mean ± SD of Surface Roughness, Colour Change and ATP

Cleaning method	n	Ra (µm), Mean ± SD	ΔE, Mean ± SD	ATP (RLU), Mean ± SD
Soap Solution	12	0.298 ± 0.045	1.65 ± 0.367	155.00 ± 32.583
Orodox Tablets	12	0.220 ± 0.032	1.18 ± 0.293	82.00 ± 20.122

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Toothsi Foam	12	0.250 ± 0.047	1.42 ± 0.314	105.00 ± 23.402
Water Control	12	0.418 ± 0.065	3.05 ± 0.458	415.08 ± 60.614
Baking Soda Paste	12	0.340 ± 0.045	2.15 ± 0.380	210.00 ± 42.074

➢ *Normality Testing*

The Shapiro–Wilk test demonstrated $p > 0.05$ for all individual group distributions for Ra, ΔE and ATP. Therefore, there was no statistically significant evidence of departure from normality.

Table 2 Shapiro–Wilk Test

Parameter	**Range of Shapiro–Wilk p-values across groups**
Surface roughness	0.975–>0.999
Colour change	0.962–>0.999
ATP	0.999–>0.999

➢ *Homogeneity of Variance*

Homogeneity of variance was satisfied for surface roughness and colour change. However, ATP showed statistically significant heterogeneity of variance ($p = 0.032$). Consequently, Welch's ANOVA and Games–Howell post-hoc analysis were used for ATP.

Table 3 Levene's Test

Parameter	**Levene's F**	**p-value**	**Interpretation**
Surface roughness	1.259	0.297	Homogeneous
Colour change	0.648	0.630	Homogeneous
ATP	2.851	0.032	Heterogeneous

➢ *Surface Roughness*

Mean surface roughness ranged from 0.220 ± 0.032 µm in the Orodox Tablet group to 0.418 ± 0.065 µm in the Water Control group.

One-way ANOVA demonstrated a statistically significant difference among the five cleaning groups:

$F(4,55) = 31.633$, $p < 0.001$.

Table 4 One-way ANOVA for Surface Roughness

Source	**Sum of Squares**	**df**	**Mean Square**	**F**	**p-value**
Between groups	0.256	4	0.064	31.633	<0.001
Within groups	0.111	55	0.002		
Total	0.367	59			

Orodox demonstrated significantly lower surface roughness than Soap Solution, Water and Baking Soda Paste. The difference between Orodox and Toothsi Foam was not statistically significant.

Table 5 Tukey HSD Pairwise Comparison of Surface Roughness

Comparison	**Mean difference**	**Adjusted p-value**
Soap – Orodox	0.078	0.002
Soap – Toothsi	0.048	0.114
Soap – Water	-0.120	<0.001
Soap – Baking Soda	-0.042	0.225
Orodox – Toothsi	-0.030	0.549
Orodox – Water	-0.198	<0.001
Orodox – Baking Soda	-0.120	<0.001
Toothsi – Water	-0.168	<0.001
Toothsi – Baking Soda	-0.090	<0.001
Water – Baking Soda	0.078	0.002

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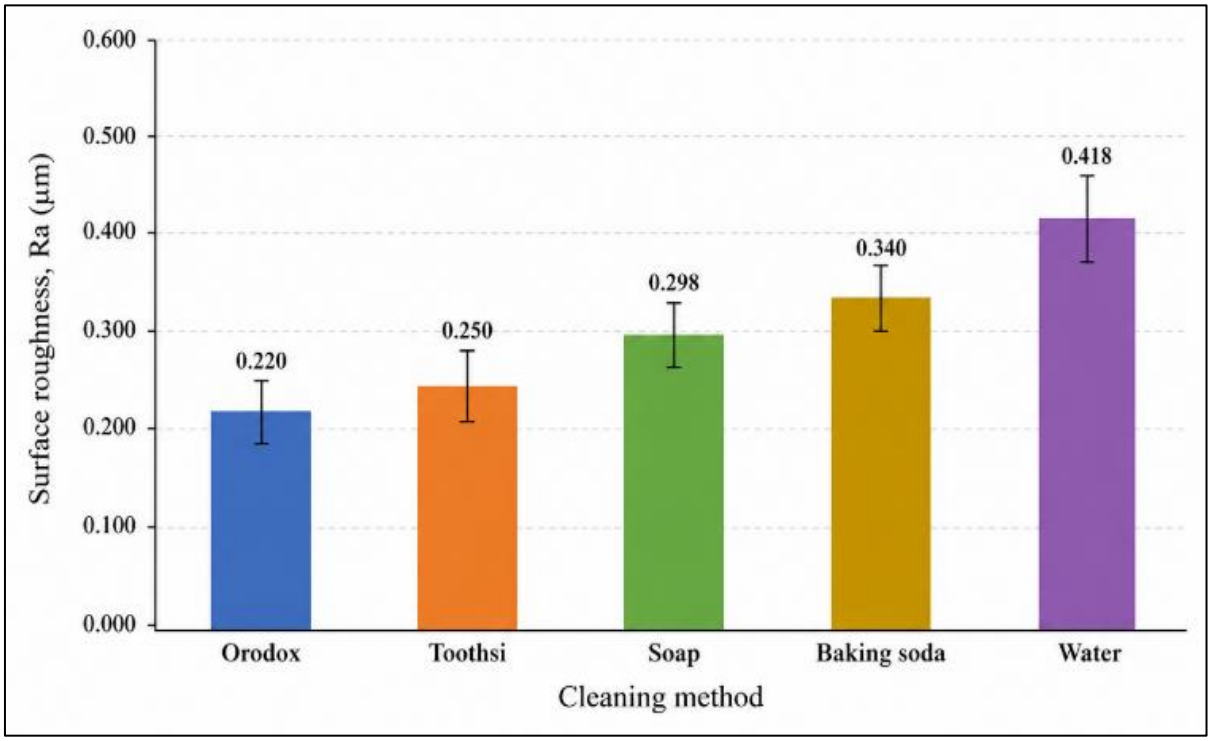

Fig 1 Mean ± SD of Surface Roughness (Ra, µm) Among the Five Cleaning Groups After 14 Days of Aligner Use.

➢ *Colour Change*

The mean colour change ranged from 1.18 ± 0.293 ΔE in the Orodox Tablet group to 3.05 ± 0.458 ΔE in the Water Control group.

One-way ANOVA demonstrated a statistically significant difference among the five groups:

$F(4,55) = 48.913$, $p < 0.001$.

Table 6 One-way ANOVA for Colour Change

Source	Sum of Squares	df	Mean Square	F	p-value
Between groups	21.964	4	5.491	48.913	<0.001
Within groups	6.172	55	0.112		
Total	28.136	59			

Orodox demonstrated significantly lower colour change than Soap Solution, Baking Soda Paste and Water. The difference between Orodox and Toothsi Foam was not statistically significant.

Table 7 Tukey HSD Pairwise Comparison of Colour Change

Comparison	Mean difference	Adjusted p-value
Soap – Orodox	0.470	0.022
Soap – Toothsi	0.230	0.545
Soap – Water	-1.400	<0.001
Soap – Baking Soda	-0.500	0.013
Orodox – Toothsi	-0.240	0.503
Orodox – Water	-1.870	<0.001
Orodox – Baking Soda	-0.970	<0.001
Toothsi – Water	-1.630	<0.001
Toothsi – Baking Soda	-0.730	<0.001
Water – Baking Soda	0.900	<0.001

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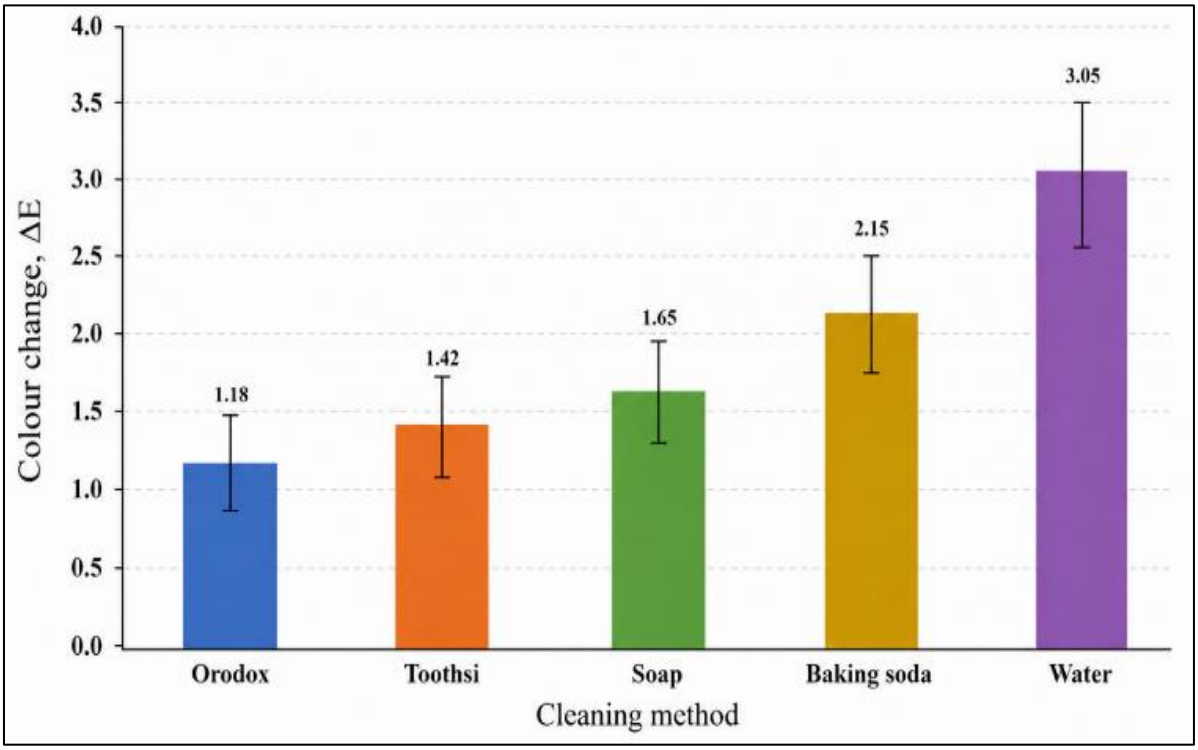

Fig 2 Mean ± SD of Colour Change (ΔE) Among the Five Cleaning Groups After 14 Days of Aligner Use.

➢ *ATP Bioluminescence*

The mean ATP value ranged from 82.00 ± 20.122 RLU in the Orodox Tablet group to 415.08 ± 60.614 RLU in the Water Control group.

As Levene's test was significant (p = 0.032), Welch's ANOVA was used.

Table 8 Welch's ANOVA for ATP

Parameter	Welch's F	df1	df2	p-value
ATP (RLU)	92.401	4	26.745	<0.001

Welch's ANOVA demonstrated a statistically significant difference in ATP values among the five cleaning groups (F = 92.401, p < 0.001).

Table 9 Games–Howell Pairwise Comparison of ATP

Comparison	Mean difference (RLU)	Adjusted p-value
Soap – Orodox	73.00	<0.001
Soap – Toothsi	50.00	0.003
Soap – Water	-260.08	<0.001
Soap – Baking Soda	-55.00	0.014
Orodox – Toothsi	-23.00	0.109
Orodox – Water	-333.08	<0.001
Orodox – Baking Soda	-128.00	<0.001
Toothsi – Water	-310.08	<0.001
Toothsi – Baking Soda	-105.00	<0.001
Water – Baking Soda	205.08	<0.001

Games–Howell analysis demonstrated significant differences between almost all pairwise comparisons. The only non-significant comparison was Orodox versus Toothsi Foam (p = 0.109).

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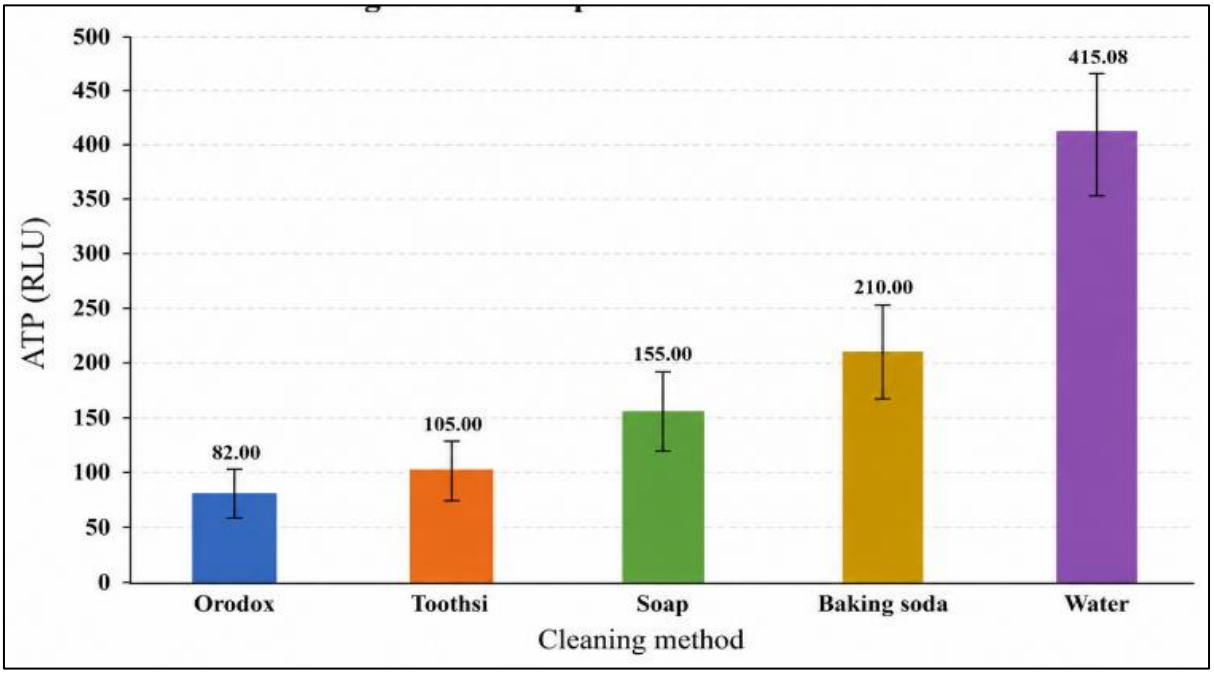

Fig 3 Mean ± SD of ATP Bioluminescence (RLU) Among the Five Cleaning Groups After 14 Days of Aligner Use.

Table 10 Overall Comparison of Study Outcomes

Parameter	Orodox Tablets	Toothsi Foam	Soap Solution	Baking Soda	Water (control)	Overall p-value
Ra (µm)	0.220 ± 0.032	0.250 ± 0.047	0.298 ± 0.045	0.340 ± 0.045	0.418 ± 0.065	<0.001
ΔE	1.18 ± 0.293	1.42 ± 0.314	1.65 ± 0.367	2.15 ± 0.380	3.05 ± 0.458	<0.001
ATP (RLU)	82.00 ± 20.122	105.00 ± 23.402	155.00 ± 32.583	210.00 ± 42.074	415.08 ± 60.614	<0.001

IV. DISCUSSIONS

The present in-vivo comparative study evaluated the effects of five patient-performed cleaning methods on clear orthodontic aligners following 14 days of intraoral use. Surface roughness, colour change and ATP bioluminescence were assessed to provide a combined evaluation of surface, optical and biological characteristics. [6,7,11,13]

The results demonstrated statistically significant differences among the five cleaning groups for all three outcome measures. Orodox Tablets demonstrated the lowest mean values for surface roughness, colour change and ATP, whereas the Water Control group demonstrated the highest mean values.

➢ *Surface Roughness*

The Orodox Tablet group demonstrated the lowest mean surface roughness (0.220 ± 0.032 µm), whereas the Water Control group demonstrated the highest (0.418 ± 0.065 µm).

One-way ANOVA showed a statistically significant difference among the five groups ($F = 31.633$, $p < 0.001$). Tukey HSD analysis demonstrated significant differences between Orodox and Soap, Water and Baking Soda groups. However, no significant difference was observed between Orodox and Toothsi.

Surface roughness is clinically important because changes in surface texture may contribute to plaque retention and staining. The literature included in the Alweneen and Alqahtani study indicates that clinical use of clear aligners may be associated with increased surface roughness and changes in material properties. [6,11]

Papadopoulou et al. previously investigated changes in roughness and mechanical properties of Invisalign appliances after clinical use. The present findings extend this concept by comparing the effect of different patient-performed cleaning protocols on surface roughness following a defined clinical usage period. [11]

➢ *Colour Stability*

The lowest mean colour change was observed in the Orodox Tablet group (ΔE = 1.18 ± 0.293), while the highest was observed in the Water Control group (ΔE = 3.05 ± 0.458).

One-way ANOVA demonstrated a statistically significant difference among the five groups ($F = 48.913$, $p < 0.001$). Tukey HSD demonstrated significant differences between Orodox and Soap, Baking Soda and Water. No significant difference was observed between Orodox and Toothsi.

Colour stability is particularly relevant to clear aligner therapy because transparency represents one of the primary aesthetic advantages of this treatment modality. The Alweneen and Alqahtani study also evaluated colour changes using spectrophotometry and reported significant differences in ΔE among cleaning methods. [13]

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The present findings therefore support the importance of considering colour stability when evaluating aligner cleaning methods.

➢ *ATP Bioluminescence*

ATP bioluminescence demonstrated the greatest separation between the cleaning groups. The Orodox group demonstrated the lowest mean ATP value (82.00 ± 20.122 RLU), while the Water Control group demonstrated the highest (415.08 ± 60.614 RLU).

Levene's test indicated unequal variances ($p = 0.032$); consequently, Welch's ANOVA was selected rather than conventional one-way ANOVA. Welch's ANOVA demonstrated a statistically significant difference among the five groups ($F = 92.401$, $p < 0.001$).

Games–Howell post-hoc analysis demonstrated significant differences between Orodox and Soap, Baking Soda and Water. Orodox and Toothsi did not differ significantly ($p = 0.109$).

The lower ATP values in the Orodox and Toothsi groups indicate lower detectable ATP-associated biological contamination under the conditions of the present assay. Conversely, the Water Control group demonstrated substantially higher ATP values.

The incorporation of ATP assessment is particularly relevant because the Alweneen and Alqahtani study did not perform microbiological assessment. Its limitations specifically state that microbiological assessments were not conducted. [13]

Thus, the present study adds a biological assessment to the surface and optical parameters investigated in the parent study. [7,13]

➢ *Comparison of Cleaning Methods*

When the three study outcomes are considered collectively, Orodox Tablets demonstrated the most favourable numerical profile.

- *Orodox Had:*

✓ The Lowest Surface Roughness;
✓ The Lowest Colour Change; And
✓ The Lowest Atp Value.

Toothsi Foam showed the second-lowest mean values for all three outcomes.

Soap Solution showed intermediate values, while Baking Soda Paste demonstrated higher values than Orodox and Toothsi.

The Water Control group showed the highest mean values for all three outcomes.

The consistent direction of these findings suggests that the cleaning method may influence multiple characteristics of the aligner simultaneously.

➢ *Comparison with the Alweneen and Alqahtani Study*

The study by Alweneen and Alqahtani evaluated five different cleaning methods: whitening toothpaste, vinegar, Fittydent Super Cleansing Tablets, Invisalign cleaning crystals and water. Twelve adult patients received five aligners, each worn for 10 days. Surface morphology was evaluated using SEM, colour change using spectrophotometry, chemical composition using FTIR and elastic modulus using nanoindentation. [13]

The present study differs in several important respects. The present study used five different patient-performed cleaning methods, included 60 participants, used a 14-day aligner usage period, assessed surface roughness using a profilometer, assessed colour change spectrophotometrically and incorporated ATP bioluminescence as a biological outcome. [13]

The Alweneen and Alqahtani study reported that Invisalign cleaning crystals maintained surface characteristics and that significant differences in ΔE were observed among cleaning methods. [13]

Therefore, although the two studies address the same broad clinical question, the present study expands the assessment to include surface roughness and ATP-based biological contamination using different patient cleaning protocols. [7,13]

➢ *Clinical Implications*

An ideal aligner cleaning method should maintain acceptable surface characteristics and colour stability while minimizing biological contamination. [6,7,11,13]

Based on the present dataset, Orodox Tablets demonstrated the most favourable overall numerical profile among the five evaluated methods.

However, these findings should be interpreted within the conditions of this study, including the 14-day aligner usage period, specified cleaning frequency and evaluated cleaning agents.

➢ *Limitations*

The present study evaluated the cleaning methods over a defined 14-day clinical period. Therefore, the findings represent short-term changes and cannot necessarily be extrapolated to prolonged aligner use. [11,13]

Patient-related factors such as oral hygiene, dietary habits, staining-agent exposure and compliance may influence aligner characteristics. [7,13]

ATP bioluminescence provides a quantitative measure of detectable biological contamination but does not identify individual microbial species. [7]

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The study also evaluated only five cleaning protocols. Further research involving larger samples, longer clinical observation and microbiological identification would provide additional evidence regarding the long-term effects of different cleaning methods. [7,13]

V. CONCLUSIONS

Within the limitations of the present in-vivo comparative study, the following conclusions were drawn:

- Statistically significant differences were observed among the five cleaning methods for surface roughness, colour change and ATP bioluminescence, with $p < 0.001$ for all three outcomes.
- The Orodox Tablet group demonstrated the lowest mean surface roughness (0.220 ± 0.032 µm).
- The Orodox Tablet group demonstrated the lowest mean colour change ($\Delta E = 1.18 \pm 0.293$).
- The Orodox Tablet group demonstrated the lowest mean ATP value (82.00 ± 20.122 RLU).
- The Water Control group demonstrated the highest mean surface roughness (0.418 ± 0.065 µm), highest colour change ($\Delta E = 3.05 \pm 0.458$) and highest ATP value (415.08 ± 60.614 RLU).
- Toothsi Foam demonstrated results broadly comparable to Orodox, and the difference between the two groups was not statistically significant for surface roughness, colour change or ATP.
- For ATP, the assumption of equal variance was violated; therefore, Welch's ANOVA followed by Games–Howell post-hoc analysis was used.
- Based on the combined assessment of surface roughness, colour stability and ATP bioluminescence, Orodox Tablets demonstrated the most favourable overall performance among the five cleaning methods evaluated.

Under the conditions of the present study, water alone demonstrated the least favourable overall profile.

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