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Original Research Article

Effects of various cleaning methods on thermoplastic orthodontic retainer material: An in vitro study

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Abstract

Background: Thermoplastic vacuum-formed retainers (VFRs) are widely used for orthodontic retention due to their aesthetics and comfort. Their mechanical and optical qualities, however, might be impacted by regular cleaning procedures. This study assessed the effect of four different cleaning techniques on the flexural modulus and light transmittance of thermoplastic orthodontic retainer materials over a 12-month simulation.

Materials & Methods: Four groups (n=14) of fifty-six specimens of thermoformed sheets (50 mm × 15 mm × 1 mm) were selected based on four different cleaning techniques: chlorhexidine mouthwash, alcohol-based mouthwash, alcohol-free mouthwash, and toothbrushing with tap water. Samples were kept at 37°C in artificial saliva and cleaned twice a week using the designated technique. A universal testing machine was used to measure the flexural modulus, and UV-visible spectrophotometry was employed to determine light transmittance at 0, 4, 8, and 12 months. The Bonferroni, and repeated measures ANOVA tests were used in the statistical analysis.

Result: Over time, all cleaning techniques produced notable changes ($p < 0.001$). The earliest and most reliable increase in flexural modulus was brought on by tooth brushing. Alcohol-based mouthwash exhibited the highest reduction in light transmittance. Mouthwashes without alcohol and chlorhexidine had relatively mild effects.

Conclusion: The physical characteristics of retainers may be negatively impacted by mechanical brushing and cleaning solutions with alcohol. Mouthwashes without alcohol or chlorhexidine provide superior retainer integrity preservation.

Keywords: Retainer cleaning, Thermoplastic retainer, Translucency, Essix retainers, Flexural modulus, Light transmittance, Orthodontic retention, Material degradation.

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1. Introduction

Orthodontic treatment necessitates a stable retention phase; thus, the quality of orthodontic retainers is crucial for maintaining corrected positions over time.[1-3] Of these, vacuum-formed retainers (VFRs) have become popular because of their superior esthetics, comfort, as compared to conventional Hawley retainers.[4-6] Their inadequate upkeep can undermine the mechanical and optical properties of thermoplastic materials, causing staining, loss of flexibility, or fracture.[7,8] Discoloured or deformed retainers can also lead to poor patient compliance and a risk of relapse.[9]

Patient cleaning techniques usually consist of either mechanical brushing or chemical disinfection with mouthwashes. Although toothbrushing is widely adopted, it may result in surface abrasions and facilitate microbial adherence.[10] Chemical agents, including chlorhexidine and solutions containing alcohol, have the potential to compromise the aesthetic properties and structural integrity of the material over time.[10,11] Specifically, alcohol-based cleaners have been demonstrated to induce a substantial reduction in light transmittance and an increase in surface roughness of retainers.[12] Similarly, exposure to chlorhexidine may elevate stiffness due to alterations in the polymer matrix. In contrast, alcohol-free cleansing agents have been observed to exert relatively limited detrimental effects.[13] Thermal forces, including cleaning retainers with hot water, can also

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warp thermoplastic appliances, which in turn affect the fit and durability.[13,14]

There is a need to determine cleaning regimens that may maintain both mechanical strength and optical clarity. This research assessed the long-term impacts of four methods of VFR cleaning—chlorhexidine mouthwash, alcohol-containing mouthwash, alcohol-free mouthwash, and mechanical brushing with tap water—on the flexural modulus and light transmittance of thermoplastic orthodontic retainers. The results will help guide clinicians in recommending evidence-based cleaning methods that provide long-term durability, aesthetics, and functionality of VFRs.

2. Materials and Methods

This in vitro study was conducted in the Department of Orthodontics at Bengaluru. The sheets of thermoplastic materials of 1mm were vacuum formed using Easy-vac EV2 vacuum forming machine. Samples were divided into four groups based on retainer cleaning methods, i.e. treatment with: Group A: Chlorhexidine mouthwash; Group B: Alcohol-based mouthwash; Group C: Alcohol-free mouthwash, and Group D: Retainers cleaned with Toothbrushing under tap water. The mouthwashes & toothbrush were procured from a local pharmacy, and artificial saliva was prepared in the Department of Biochemistry.

2.1. Specimen preparation

The clear thermoforming retainer material was first processed over a stainless-steel block with the following dimensions: 55mm x 18mm x 6mm, using the Easy-vac EV2 vacuum forming machine (**Figure 1**A). The samples were cut from the processed sheet into the standard dimensions of 50mm x 15mm x 1mm (**Figure 1**B) using a diamond saw. Fifty-six specimens of each prepared material were randomly divided into four groups (n =14) for each cleaning solution and the toothbrushing group. Each group consisted of fourteen samples, and all were stored at 37°C in artificial saliva in containers appropriately labelled inside the incubator. The specimens in each group were tested for flexural modulus and light transmittance at 0 months (T0), 4 months (T4), 8 months (T8) & 12 months (T12).

2.2. Artificial saliva preparation

Artificial saliva was prepared with 1.6g sodium chloride, 1.6g potassium chloride, 3.18g calcium chloride dehydrate, 2.76g sodium dihydrogen phosphate monohydrate, 0.02g sodium sulfide nonahydrate, 4g urea, and 4000 mL distilled water. The saliva was thoroughly mixed overnight on a magnetic stir plate. The day following saliva preparation, potassium hydroxide was added to the saliva to achieve a final pH of 6.75.

2.3. Experimental process

Throughout the study period, specimens remained in artificial saliva at 37°C. Twice a week, each group of specimens was removed from the artificial saliva, rinsed thoroughly with water, and then specimens of Groups A, B & C were immersed in the appropriate cleaning solution for 15 minutes. While specimens of Group D were brushed with tap water. (**Figure 2**)

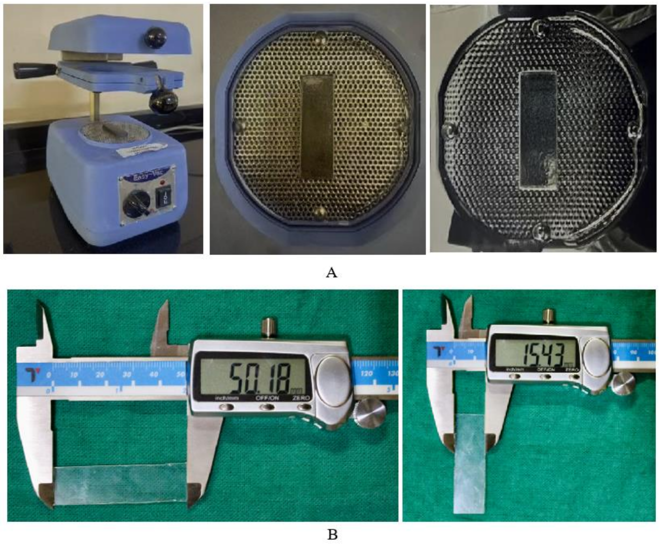

Figure 1: A): Thermoforming of clear retainer sheets; **B):** Thermoformed plastic material after being cut to the required dimensions

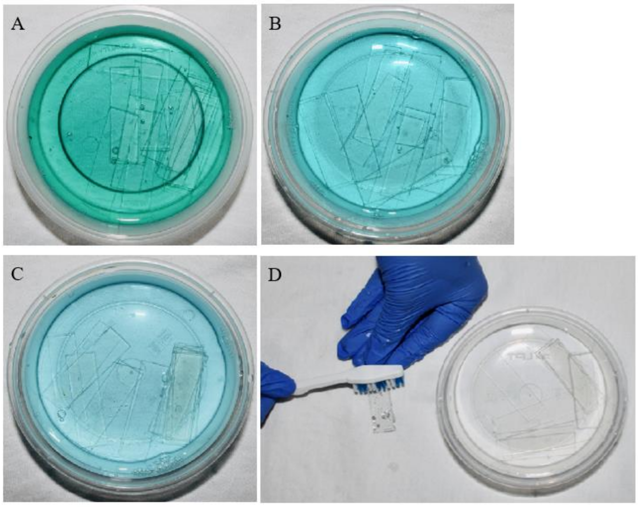

Figure 2: Specimens from Groups A, B, C, and D after treatment with their respective cleaning methods; **A**): Group A: Soaked in chlorhexidine mouthwash; **B):** Group B: Soaked in alcohol-based mouthwash; **C)**: Group C: Soaked in alcohol-free mouthwash; **D**): Group D: Brushed manually under tap water

2.4. Data collection

The two physical properties that were measured for each of the specimens tested included: a) Light transmittance and b) Flexural modulus.

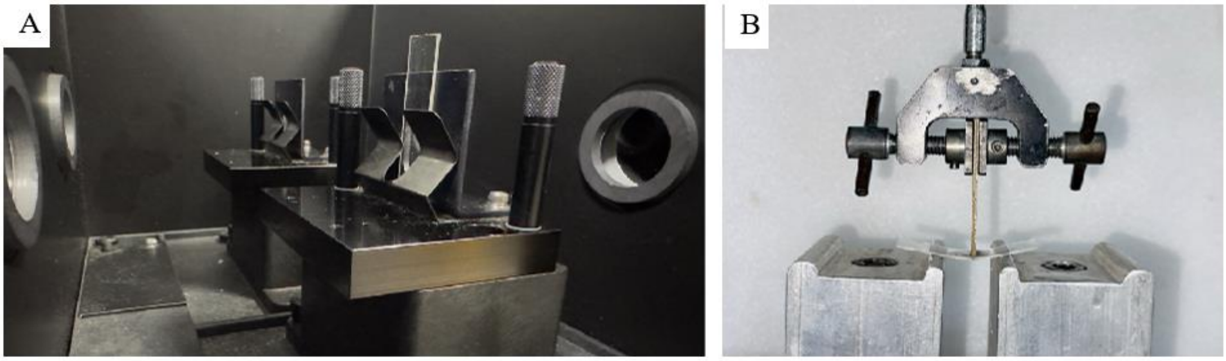

Figure 3: Specimen testing procedures: **A):** Light transmittance test conducted using UV spectrometer; **B):** Flexural modulus evaluation using a universal testing machine with a three-point bending setup

2.5. Light transmittance

Light transmittance was performed with a double-beam pathway UV/visible spectrophotometer (LAMBDA 1050+, PerkinElmer Inc., Waltham, USA) (**Figure 3**A). After the specimen was placed in the sample cell compartment, the light beam from the source was passed through the monochromator, and then the light beam was split into a double beam with the beam splitter; each beam was passed through one of two sample compartments: the reference cell compartment containing air and the sample cell compartment containing the specimen. The T% of each wavelength, from 380 nm to 740 nm, was measured automatically after being calibrated and integrated in UV Win Lab Software through division of the intensity of the light leaving the sample (I) by the intensity of the light entering the sample. Finally, the overall T% for each specimen was calculated as the average for all integrated T%.

2.6. Flexural modulus

A universal testing machine (MultiTest 10-i) was used to conduct a three-point bend test. (**Figure 3**B) Prior to starting any testing, each individual specimen was measured using calipers for width and thickness respectively. The specimen was held between two blocks in order to center itself on the fixture. The span length was set at 24 mm, which was calculated as the sum of the average widths of the 2 maxillary central incisors and 1 lateral incisor. The plunger was lowered using the down arrow on the remote until it just touched the sample. The center of the specimen was deflected vertically 2mm at a rate of 1mm per minute; connected to a 100-N load cell. The values were derived in Newtons (N) and then using the following formula was converted into Megapascals (MPa).

$$E_{flex} = \frac{L^3 F}{4wh^3 d}$$

Where,

E= Flexural modulus

L = Length of span

F = Force

w = width of sample

h = thickness of sample

d = deflection

2.7. Statistical analysis

Within this experimental framework, the following hypothesis was proposed: ***Null Hypothesis (*** H_0 ***):*** There is no significant difference in the flexural modulus and light transmittance of thermoplastic vacuum-formed retainers (VFRs) subjected to different cleaning methods over time, and ***Alternative Hypothesis (*** H_1 ***):*** There is a significant difference in the flexural modulus and light transmittance of thermoplastic vacuum-formed retainers (VFRs) subjected to different cleaning methods over time.

Flexural modulus was analyzed by the repeated measures ANOVA test in all groups (the p-values being less than 0.001* suggests that the changes observed are not due to random chance).

3. Results

This in vitro study evaluated the effects of four cleaning methods—chlorhexidine (CHX) mouthwash, alcohol-based mouthwash, alcohol-free mouthwash, and toothbrushing under tap water—on the flexural modulus (mechanical property) and light transmittance (optical property) of thermoplastic orthodontic retainers (Samples: Four groups; n=14 per group) over 12-months at time points: Baseline (T0), 4 months (T4), 8 months (T8), 12 months (T12).

3.1. Flexural modulus

Flexural modulus values increased significantly over time within all four groups (**Table 1**). The greatest increase was observed in the Chlorhexidine (CHX) and Toothbrushing groups. Repeated measures ANOVA confirmed these changes were statistically significant across all groups ($p < 0.001$). These results suggest that all cleaning methods increase material stiffness over time. Post hoc Bonferroni analysis (**Table 2**) showed significant differences from baseline beginning at T8 for CHX, AL, and ALF groups ($p < 0.05$). The TB group exhibited significant differences from as early as T4.

One-way ANOVA (**Table 3**) revealed no significant differences in flexural modulus between groups at T0, T4, or T12 (p > 0.05). However, at T8, a statistically significant difference was observed (p = 0.029), with the CHX group exhibiting higher modulus values than the AL group.

Table 1: Comparison of the change in flexural modulus (MPa) within each group

Interval	CHX Mean ± SD	Alcohol-based Mean ± SD	Alcohol-free Mean ± SD	Toothbrushing Mean ± SD	p-value
T0	1441.89±100.24	1437.50±58.89	1449.05±90.45	1439.49±96.00	<0.001*
T4	1547.71±105.97	1475.67±68.88	1481.47±119.82	1521.71±110.91	<0.001*
T8	1724.38±114.45	1576.76±155.20	1610.41±128.62	1644.56±124.20	<0.001*
T12	1927.87±170.47	1839.91±150.02	1847.73±130.35	1906.81±79.68	<0.001*

Repeated measures ANOVA test; * indicates a significant difference at p≤0.05

Table 2: Pairwise comparison of the change in flexural modulus (MPa) from baseline (T0) within each group

Interval	CHX	Alcohol-based	Alcohol-free	Toothbrushing
T0-T4	0.152	0.118	1.000	0.046*
T0-T8	<0.001*	0.046*	0.010*	0.003*
T0-T12	<0.001*	<0.001*	<0.001*	<0.001*

Post hoc Bonferroni test; * indicates a significant difference at p≤0.05

Table 3: Inter-group comparison of flexural modulus (MPa) between four groups

Interval	F-value	p-value	Notable findings
T0	0.127	0.987	NS between groups at baseline
T4	1.878	0.216	NS at 4 months
T8	3.242	0.029*	Significant; CHX > Alcohol-based (p=0.023)
T12	1.410	0.250	NS at 12 months

One-way ANOVA test; Significant difference at p≤0.05; *NS = Not significant

3.2. Light transmittance

Comparison of the change in light transmittance (T%) within each group showed a consistent and statistically significant decrease in transmittance was observed within all groups over time (p < 0.001), indicating increased opacity (**Table 4**). The AL group showed the most pronounced reduction in clarity, with statistically significant changes beginning from T4. CHX showed significant change from T8 onward. Within each group, the change in light transmittance (T%) from baseline was evaluated using post hoc Bonferroni testing (**Table 5**).

1. Alcohol-based mouthwash, alcohol-free mouthwash, and toothbrushing under tap water all caused a statistically significant reduction in transmittance starting as early as 4 months, with this effect maintained through later time points.
2. CHX (chlorhexidine) mouthwash showed a significant decrease in transmittance only after 8 months, with significance continuing at 12 months.

This indicates that, compared to CHX, the other cleaning methods lead to earlier and more sustained reductions in retainer optical clarity.

Among the four experimental groups at baseline (T0), there were no statistically significant differences in mean transmittance (T%) (**Table 6**). However, by T4, the Alcohol-based mouthwash group exhibited a markedly lower mean transmittance, with statistically significant differences observed across all groups. This trend persisted at T8, where the Alcohol-based group continued to demonstrate the lowest transmittance values, while the Alcohol-free group maintained the highest optical clarity. By T12, the Alcohol-based mouthwash group recorded the most pronounced reduction in transmittance, indicating the greatest loss in transparency. The Toothbrushing group followed with a notable decrease, whereas the CHX and Alcohol-free groups showed comparatively minor but statistically significant reductions. The intergroup differences in transmittance values became highly significant by the 12-month mark.

4. Discussion

This research focused on the long-term impact of four prevalent cleaning methods - mouth rinsing with chlorhexidine, mouth rinsing with alcohol, non-alcohol mouth rinsing, and brushing with tap water - on the flexural modulus and light transmittance of thermoplastic vacuum-formed retainers (VFRs) over a simulated period of 12 months. These properties are important retentive, functional, and aesthetic qualities of orthodontic retainers.

Table 4: Comparison of the change in transmittance (T%) within each group

Interval	CHX Mean ± SD	Alcohol-based Mean ± SD	Alcohol-free Mean ± SD	Toothbrushing Mean ± SD	p-value
T0	88.81±1.82	89.70±1.81	90.26±1.47	90.10±1.18	<0.001*
T4	86.88±1.53	80.36±3.77	87.25±1.93	86.04±2.57	<0.001*
T8	84.81±1.73	77.73±2.33	86.50±1.29	81.03±2.26	<0.001*
T12	83.77±2.41	71.39±2.09	84.41±1.36	77.79±1.47	<0.001*

Repeated measures ANOVA test; * indicates a significant difference at p≤0.05

Table 5: Pairwise comparison of the change in transmittance (T%) from baseline (T0) within each group

Interval	CHX	Alcohol-based	Alcohol-free	Toothbrushing
T0-T4	0.162	<0.001*	0.007*	<0.001*
T0-T8	0.001*	<0.001*	<0.010*	<0.001*
T0-T12	<0.001*	<0.001*	<0.001*	<0.001*

Post-hoc Bonferroni test; * indicates a significant difference at p≤0.05

Table 6: Comparison of transmittance (T%) between four groups

Interval	F-value	p-value	Notable findings
T0	2.35	0.083	NS at baseline
T4	21.59	<0.001*	Alcohol-based lower than others
T8	56.77	<0.001*	Significant between groups
T12	146.15	<0.001*	Significant between groups

One-way ANOVA test; Significant difference at p≤0.05; *NS = Not significant

4.1. Flexural modulus

The flexural modulus indicates the stiffness of the material and its resistance to bending. Higher modulus implies higher stiffness, which can influence the retainer's fit and comfort. After moistening, all four cleaning procedures led to a significant time-dependent gain in flexural modulus (p < 0.001), which reflected the progressive hardening of the material, regardless of the cleaning method chosen.

The earliest and greatest rise was after mechanical toothbrushing when changes were evident by 4 months postbrushing (T4). This might be attributed to the abrasive nature of the brushing, human power and microcracks and surface damage introduced, leading to increased stress concentration and reduced flexibility, also seen in a study by Wible et al. Slow onset was observed with chemical processes. CHX, alcohol-based BMs, and alcohol-free BMs showed an increase in flexural modulus of statistical significance starting from 8 months (T8), indicating that interaction with chemical/chelating acid of a long-term nature with the polymer matrix caused the decomposition of internal bonds.

There was a considerable higher modulus of the CHX group compared to the alcohol based group at T8 (p = 0.023). This is in agreement with Hussein et al., who demonstrated that CHX affects the organization of the polymer chains in thermoplastics, leading to an increase in the brittlness of the material with time. Although CHX and alcohol-based materials show good efficacy in disinfection, the long-term use of CHX and alcohol-based products can diminish retainer flexibility and, consequently, their adaptation and patient comfort.

Alcohol-free mouthwash had the most conservative alterations, and was therefore the least aggressive among those tested. This is consistent with the results of Wible et al., who promoted the safety of non-alcohol formulas on retainer integrity compared to other findings.

4.2. Light transmittance

The light transmittance (T%) is one of the most important optical properties in the transparency and esthetic appearance of VFRs. A decrease in T% indicates greater opaqueness or discoloration, which could decrease patient acceptance. Transmittance in all groups decreased significantly over time (p < 0.001).

The alcohol-containing mouthwash cohort demonstrated a rapid and early reduction, with significant reductions from T4 to T12 (20.7%). The solvent action of alcohol breaks polymer-polymer bonds, leading to the increase in surface roughness and light scattering, with an associated loss of clarity. Agarwal et al as well also noted discoloration and reduced light penetration on polyurethane retainers exposed to alcohol-based cleaners.[11]

CHX and alcohol-free mouthwashes also produced a decrease in transmittance; however, the effects were less intense and reached statistical significance only from T8. Although CHX is used as an antimicrobial, over time it may induce subtle molecular alterations, which can result in mild turbidity.

Toothbrushing with tap water also caused a decrement of transparency with time. Though it is generally considered as a safer technique, repeated mechanical wear from brushing can generate microgrooves as well as surface defects in the in the former case, so that abrasion is responsible of the increase of the opacity.

The findings are similar to those reported by Wible et al., and Hussein et al., who reported a high relation of surface roughness and light transmittance, especially in retainer materials that underwent physical wear or chemical wear.[8,10] Patients can be distressed by the appearance of retainers that show visible staining or are opaque how this can reduce patient compliance and consequently their post-treatment stability.

4.3. Integration with literature

The findings of this study are supported by a broad body of literature examining the behavior of thermoplastic orthodontic materials under various environmental stressors. Studies from 2000 onwards have consistently reported that exposure to fluctuating temperatures, moisture, UV radiation, and chemical agents contributes to the degradation of mechanical and aesthetic properties in materials such as polyurethane, copolyester, and polypropylene.

For example, Ryokawa et al. and Susarchick et al. reported that the intraoral environment, with its constant exposure to saliva, enzymes, and temperature fluctuations, accelerates material fatigue, leading to a loss in elasticity and transparency.[14,16] Additional studies using SEM and spectrophotometry have shown that surface degradation is often accompanied by internal molecular disorganization, supporting the observed trends in this study.

Notably, a study by Hussein et al. evaluated seven different cleaning agents and confirmed that substances like vinegar, sodium hypochlorite, and hydrogen peroxide lead to increased surface roughness and reduced light transmittanc.[8]

This present study builds on that knowledge by offering a controlled comparison of four widely used cleaning agents over a longer period (12 months), thereby contributing novel insights to clinical orthodontic practice.

5. Clinical Relevance and Recommendations

On the basis of clinical practice, the present study demonstrates that not all cleaning methods can do without secondary side-effects. Alcohol-based solutions are good disinfectants, but are most deleterious to esthetic properties, and thus should be carefully applied and not for long durations. Toothbrush application is simple and readily available, but can reduce mechanical properties and result in premature stiffness and decreased clarity.[10]

Of all techniques, alcohol-free mouthwash proved to be the most "balanced" method, with average actions on both mechanical and optical parameters. In patients demanding esthetics or using VFRs for long periods, the clinician should consider advising an alcohol-free or lightly antiseptic solution for daily cleaning.

Patient education is also crucial. These things can cause damage to of course the retainers that people don’t realize. Proffit et al. and Graber et al. warning against thermal stress, that may distort the material and interfere with the fit.[13,15]

Regular evaluations of retainers should be conducted to check for wear, discoloration, and distortion when retainers also need to be replaced on a function and esthetics basis rather than a specific date.

5.1. Limitations and future directions

The present research is an in vitro study, and the replication of complexity of intraoral situation such as enzymatic activity, fluctuation of temperature and masticatory forces effect real life retainer wear; have not been fully simulated. Furthermore, only 2 parameters were studied as there are still other factors such as microbial adhesion, tensile strength, surface roughness that deserve to be explored.

Further in vivo models, additional thermoplastics and investigation of novel cleaning techniques (UV disinfection, ultrasonic cleaning) are to be studied. New material formulations that are resistant to chemical and mechanical degradation may also increase the success of long-term retention.

6. Conclusion

Within the limitations of this study, the following conclusions were drawn:

1. All four cleaning methods—chlorhexidine mouthwash, alcohol-based mouthwash, alcohol-free mouthwash, and toothbrushing under tap water—caused statistically significant changes in flexural modulus and transmittance (T%) of thermoplastic vacuum-formed retainers (VFRs) over 12 months.
2. Alcohol-based mouthwash resulted in the most rapid and pronounced reduction in transmittance, indicating early and significant loss of optical clarity.
3. Toothbrushing under tap water caused the earliest and most progressive increase in flexural modulus, reflecting enhanced material stiffness due to mechanical abrasion.
4. Chlorhexidine mouthwash induced a significant increase in flexural modulus from the eighth month onward.
5. Alcohol-free mouthwash produced the least aggressive changes, showing moderate effects on both mechanical and optical properties.

The study shows that patients should be advised to use milder, non-abrasive cleaning agents to preserve the structural and esthetic integrity of retainers. Further in vivo studies are recommended to assess long-term clinical implications, including effects on surface roughness, microbial colonization, and alternative retainer materials or cleaning technologies.

7. Source of Funding

None.

8. Conflict of Interest

None.

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