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Comparative Effects of Thermocycling and Artificial Saliva Immersion on Surface Roughness and Light Transmittance of 3D-Printed and Thermoformed Orthodontic Aligners: An In-Vitro Study

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Abstract

Aim
This in vitro study compared the effects of thermocycling and artificial saliva immersion on the light transmittance and surface roughness of thermoformed (TF) and directly 3D-printed (DP) orthodontic aligners.

Methods
Twenty-four aligners were used: 12 multilayer thermoplastic aligners (CA® Pro+, Scheu Dental) and 12 directly 3D-printed resin aligners (Tera Harz TC-85, Graphy). Each material was divided into three independent subgroups of four specimens: as-received (AR), after 500 thermocycles (5 °C to 55 °C), and after 14 days of immersion in artificial saliva at 37 °C (AS). Light transmittance (T%) was measured with a UV/Vis spectrophotometer over 400–700 nm, and surface roughness (arithmetic mean height, Sa) was assessed by atomic force microscopy over a 2 × 2 µm area. Between-material comparisons used independent-samples t-test, and comparisons among the three conditions within each material used one-way ANOVA with Tukey post hoc tests ($\alpha = 0.05$).

Results
Baseline T% did not differ significantly between TF and DP aligners (95.79 ± 1.94% vs. 87.26 ± 7.38%; $P = 0.067$). After thermocycling, T% was 52.37 ± 19.23% for TF and 10.49 ± 4.48% for DP aligners ($P = 0.005$). After saliva immersion, T% was 65.33 ± 5.83% for TF and 12.45 ± 7.35% for DP aligners ($P < 0.001$). Sa after thermocycling was 23.16 ± 13.15 nm for TF and 70.99 ± 18.12 nm for DP aligners ($P = 0.005$). After saliva immersion, Sa was 25.44 ± 23.15 nm for TF and 52.47 ± 5.51 nm for DP aligners ($P = 0.063$). Within TF aligners, T% ($P = 0.001$) and Sa ($P = 0.02$) differed among conditions. Within DP aligners, T% differed ($P < 0.001$), but Sa did not ($P = 0.14$). All mean Sa values were below 500 nm.

Conclusion
Under these short-term aging conditions, thermoformed (TF) aligners had higher light transmittance and lower surface roughness than directly 3D-printed (DP) aligners after thermocycling. Because each aging condition was applied to an independent subgroup of four specimens, the sample is small, and aligner thickness differed between materials, these findings are exploratory and should be interpreted with caution.

Keywords: 3D printing, Orthodontic Aligners, Spectrophotometry, Surface Roughness, Thermocycling

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Background

The high demand for aesthetic clear aligners (CAs) is tempered by the cost and complexity of third-party fabrication. In-office 3D printing presents a cost-effective, controlled solution for same-day delivery, yet concerns persist regarding the resulting materials' properties, aging, and reproducibility (1,2).

The three primary vat photopolymerization technologies—stereolithography (SLA), digital light processing (DLP), and liquid crystal display (LCD)—differ principally in their light source and layer-curing method. Inconsistent printing parameters (e.g., build orientation, layer thickness, and technology type) and variable post-processing conditions (e.g., curing time, temperature, and cleaning methods) can compromise fabrication accuracy and lead to non-uniform surface characteristics (3,4).

Material transparency, an optical property determined by light transmittance percentage (T%) and absorbance, is a critical aesthetic attribute that reduces aligner visibility and meets patient demand for discreet orthodontic treatment. High transmittance yields greater transparency, while high absorbance increases opacity (5). This feature has become a key marketing focus for industry leaders like Invisalign and a primary driver in developing new aligner materials, which must balance aesthetic requirements with necessary mechanical performance.

Thermoplastic aligner materials are inherently reactive to the oral environment. Temperature fluctuations, humidity, saliva, and mechanical loading can significantly alter their properties. Although in vitro testing methods cannot fully replicate clinical conditions, the effect of water absorption—and its associated hygroscopic expansion—remains a critical mechanical consideration (3,6,7). Furthermore, the thermoforming process itself, which involves a heating cycle under pressure or vacuum, alters the material's morphological and mechanical integrity. Subsequent intraoral use subjects the material to additional thermal, mechanical, and chemical degradation, often manifesting as visible surface alterations and color changes (6,7).

Atomic force microscopy (AFM) enables assessment of surface quality by comparing mean surface roughness before and after aging, with or without thermocycling (6,8,9). Previous studies indicate that aging significantly alters the surface characteristics of clear aligners (7,10,11). This study employed thermocycling to investigate the clinical endurance of clear aligners. To account for physicochemical degradation from water absorption, aligners were stored in artificial saliva and subjected to thermocycling (9). This combined approach assessed the durability and stability of commercially available thermoplastic and 3D-printed materials under simulated oral conditions.

Several recent studies have investigated the optical or surface behavior of orthodontic aligners, but important differences remain in their materials, aging protocols, and outcome measures. Cremonini et al. (5) evaluated light transmittance of aligners produced with different 3D printers and thermoforming materials, after 14 days of aging in artificial saliva; however, they did not evaluate directly 3D-printed aligners or nanoscale surface roughness. Koletsi et al. (11) compared surface roughness between retrieved Invisalign and in-house 3D-printed aligners but did not assess light transmittance or standardized thermocycling and artificial-saliva aging. Šimunović et al. (12) compared thermoformed and 3D-printed polyurethane aligners for beverage-induced color and chemical changes using colorimetry and ATR-FTIR spectroscopy, rather than light transmittance and nanoscale surface roughness under controlled aging conditions. More recently, Neoh et al. (3) compared thermoplastic materials and 3D-printing resins under dry, wet, and thermocycled conditions and evaluated both surface and optical properties; however, they used different materials and standardized clear retainer specimens, with 10,000 thermocycles rather than the 500-cycle protocol used in the present study.

This study aimed to compare the effects of thermocycling and artificial saliva immersion on the light transmittance and surface roughness of directly 3D-printed versus thermoformed orthodontic aligners.

Methods

Study Design

This in vitro study used the individual aligner specimen as the unit of analysis. Each aligner was measured once for each outcome (surface roughness and light transmittance); no repeated measurements were taken on the same specimen.

Sample Size Estimation

A sample size calculation was performed using G*Power (version 3.1.9.7) for a one-way ANOVA (fixed effects, omnibus) with three independent groups (as-received, thermocycling, and artificial saliva) within each aligner material. Assuming a large effect size (Cohen's f = 1.20), a power (1−β) of

0.80, and an α of 0.05, the minimum required sample size was 4 specimens per group.

Aligner Materials

The sample consisted of 24 aligners from two brands:

- 12 Graphy aligners (Graphy Inc., Seoul, Korea). Graphy aligners are made from Tera Harz TC-85 DAC resin, a shape-memory material used to manufacture direct 3D-printed aligners (Figure 1A).

- 12 CA Pro+ aligners (Scheu Dental; Iserlohn, Germany). Scheu CA Pro+ is a tri-layer thermoplastic polyurethane material (Figure 1B).

Scheu CA Pro+ aligners are thermoformed CAs, whereas GRAPHY aligners are direct 3D-printed orthodontic aligners. To expose the facial surface, the anterior lingual part of each aligner was removed using scissors. The maxillary right central incisors from the aligners were chosen for sample preparation because of their relatively flat shape, which allows for more precise observations of surface topography using an atomic force microscope.

Fabrication and Specimen Preparation

The maxillary arch typodont model was scanned using the Shining 3 D Aoralscan 2 intraoral scanner (China). 3D-printed aligners were digitally fabricated using AccuDesign (Pune, Maharashtra, India) and AccuWare (Glen Ellyn, Illinois, USA) software, and the designs were saved in standard tessellation language (STL) file format. The aligners were directly printed using an AccuFab-L4K 3D printer (SHINING 3D®, China), which employs LCD-based mask stereolithography (MSLA) technology, with a layer thickness of 50 µm and an oblique build orientation at 45° to mitigate additive manufacturing defects. Additionally, the overall thickness of the Graphy aligners was set to 0.5 mm to accommodate the expected decrease in CA Pro+ (0.75 mm) during the pressure-molding process. After printing, the support structures were excised with a scraper, and the aligners were post-processed as per the manufacturer's instructions.

We designed templates with the required dimensions for the thermoplastic samples using the same software. Thermoplastic sheets were then pressure-thermoformed over the 3D-printed models in a MINISTAR S® pressure-molding unit (Scheu-Dental, Germany) following the manufacturer's recommended settings: heating temperature: 220°C, heating time: 30 s, cooling time: 60 s, evacuation time: 7 s, and chamber pressure: 4 bar. After cooling, the pressure-formed sheets were trimmed to the appropriate dimensions for each testing procedure.



Figure 1: (A) A direct 3D-printed aligner fabricated from Tera Harz TC-85 DAC resin (B) A multilayer aligner made from thermoplastic polyurethane (CA Pro+).

Excess uncured resin was removed from the 3D-printed aligners by centrifugation at 600 rpm for 6 minutes. The aligners were then ultrasonically cleaned for 3 minutes and air-dried on paper towels for 5 minutes. Subsequently, they were cured in a Graphy Tera Harz Cure THC 2 UV-curing unit under a nitrogen atmosphere at Level 2 for 20 minutes.

Simulated Oral Aging Protocols

Thermocycling was performed to accelerate aging according to ISO/TS 11405:2015. Each cycle consisted of a 20-s dwell time in a cold bath at 5±2°C and a 20-s dwell time in a hot bath at 55±2°C, with a maximum transfer time of 10 s between baths. A total of 500 cycles were applied. Each aging group comprised 4 Graphy® and 4 CA® Pro+ aligners.

In addition, 4 Graphy® and 4 CA® Pro+ aligners were stored in separate glass containers filled with artificial saliva and incubated at 37°C for 2 weeks. The artificial saliva was prepared according to ISO/TR 10271:2020 and contained (per liter): 0.4 g NaCl, 0.4 g KCl, 0.795 g CaCl2·2H2O, 0.78 g NaH2PO4·2H_2O, 0.005 g Na2S·9H2O, and 1.0 g urea, with the pH adjusted to 6.8±0.1.

Specimens were allocated to three mutually independent subgroups per material (n = 4 each): as-received (AR, no aging), thermocycling (TC, measured immediately after 500 cycles), and artificial saliva (AS, measured after 14 days of immersion at 37 °C). Each specimen underwent only one condition and was measured once; no specimen was reused across conditions.

Surface Roughness Characterization (AFM)

Surface topography was assessed at the nanoscale using an atomic force microscope (AFM) operating in tapping mode (Figure 2A). Scans were collected from the central region of the facial surface of the maxillary right central incisor, away from any trimmed margins. One 2 × 2 µm area was scanned per specimen , and three-dimensional topographic images were generated. Surface roughness was quantified using the arithmetic mean height (Sa) as the single predefined outcome. Sa was chosen because it is the most widely reported amplitude parameter in the aligner and dental polymer literature, which allows direct comparison with previous studies, and because it is less affected by isolated peaks or pits than extreme-value parameters. Other amplitude parameters were not analyzed: the root mean square height (Sq) is mathematically closely related to Sa and adds little independent information, whereas the maximum peak height (Sp), maximum pit height (Sv) and maximum height (Sz) depend on single extreme points and are therefore unstable in small scan areas (2 × 2 µm) and small samples (n = 4).

Optical Transmittance Measurement (UV-Vis)

Light transmittance was expressed as percentage light transmittance (T%), representing the percentage of incident light transmitted through the aligner material. A higher T% indicates greater light transmission and, consequently, greater aligner transparency. T% and absorbance were measured using a UV/Vis spectrophotometer (LAMBDA™ 365, PerkinElmer Inc., USA). Each aligner specimen was placed in a quartz cuvette holder, and T% was recorded over the visible spectrum.

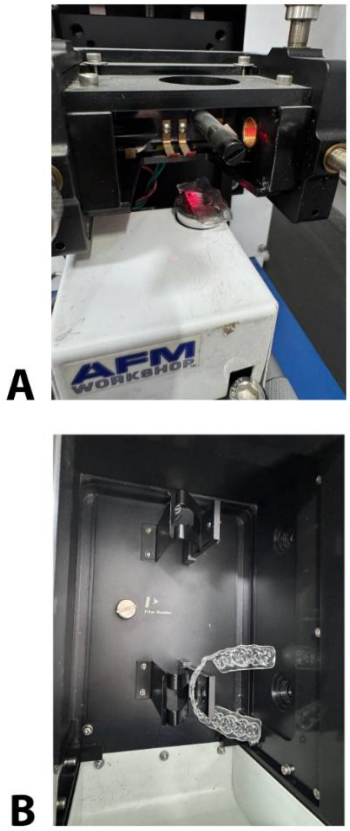


Figure 2:
(A) An aligner sample mounted on a stub for surface roughness analysis using an atomic force microscope in tapping mode.
(B) A sample positioned in a quartz cuvette holder within the sample compartment of a UV-Vis spectrophotometer to ensure a consistent optical path length for light transmittance measurements.

Statistical Analysis

Data were analyzed using version 26 of IBM® SPSS® software (SPSS Inc., IBM, Chicago, USA). Shapiro–Wilk and Levene's tests were used to assess normality and homogeneity of variances. Descriptive statistics included means and standard deviations (SDs), together with statistical tables and figures. For direct comparisons between the two independent materials under the same condition, independent-samples t-tests were employed. Comparisons between measured as-received (AR) thermocycling (TC) and artificial saliva(AS) within each material were performed using independent-samples t-tests. P-value<0.05 was considered as significant.

Results

Light transmittance: Shapiro–Wilk testing confirmed normal distribution of the data (P>0.05). Mean T% values (where higher T% indicates greater transparency) for TF aligners exceeded those for DP aligners in the as-received, artificial saliva, and thermocycling conditions. For Thermoformed aligners, T% was 95.79±1.94% as-received, 52.37±19.23% after thermocycling, and 65.33±5.83% after artificial saliva immersion.

For 3D-printed aligners, T% was 87.26±7.38% as-received, 10.49±4.48% after thermocycling and 12.45±7.35% after artificial saliva immersion. Independent-samples t-tests demonstrated a statistically significant difference between TF and DP aligners after thermocycling (P=0.005) and after immersion in artificial saliva (P<0.001), whereas the difference in the as-received condition was not statistically significant (P=0.067).(Figure 3)

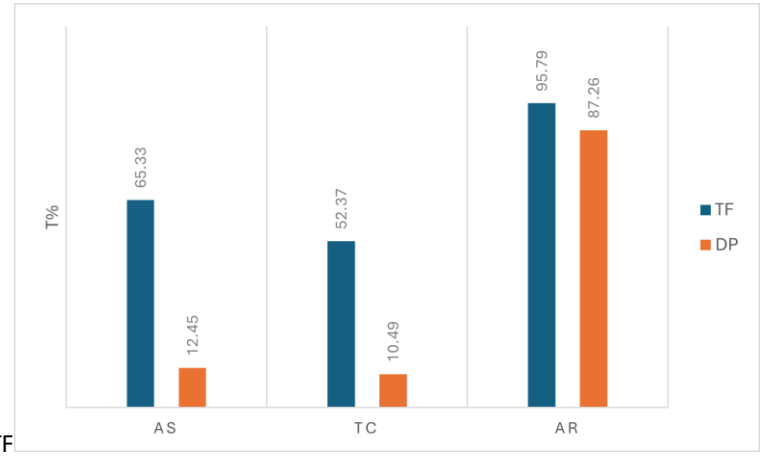
The one-way ANOVA statistical test for multilayer thermoplastic aligners revealed a significant difference between at least one of the three groups (TC, AS, and AR)(p=0.001), and the Tukey post-hoc test confirmed a difference between the AS and AR subgroups ($p < 0.05$).

Furthermore, a one-way analysis of variance (ANOVA) of the resin aligners—fabricated directly via 3D printing—revealed a significant difference among at least one of the three groups (TC, AS, and AR) ($p < 0.001$). A subsequent Tukey post-hoc test confirmed a significant difference between the AS and AR subgroups ($p < 0.05$).

Surface roughness: The assumption of homogeneity of variance was assessed using Levene's test. Mean Sa values for TF aligners were lower than those for DP aligners after thermocycling and immersion in artificial saliva. After thermocycling, Sa was 23.16±13.15 nm for TF and 70.99±18.12 nm for DP aligners and the difference was statistically significant (P=0.005). After immersion in artificial saliva, Sa was 25.44±23.15 nm for TF and 52.47±5.51 nm for DP aligners, with no statistically significant difference between the materials (P=0.063). In the as-received condition, the difference between TF (63.72±19.35 nm) and DP (65.45±9.93 nm) was not statistically significant (P=0.879) (Figure 4 &Table 1).

In multilayer thermoplastic aligners The one way ANOVA test showed statistically significant difference in Sa between SA,TC,AR (p=0.02). And this difference between AS and AR was significant ($p < 0.05$).

However, for resin aligners—produced directly via 3D printing—no significant difference was observed among the three conditions (TC, AS, and AR) ($p = 0.14$).



TF

Figure 3: Bar chart comparing light transmittance (T%) between TF and DP aligners after AS, TC, and AR conditions.
Mean ± SD values: TF AS 65.33 ± 5.83%; DP AS 12.45 ± 7.35%; TF TC 52.37 ± 19.23%; DP TC 10.49 ± 4.48%; TF AR 95.79 ± 1.94%; DP AR 87.26 ± 7.38%.
T%: transmittance percentage
TF: thermoformed
DP: directly printed
AR: as-received
AS: artificial saliva
TC: thermocycling

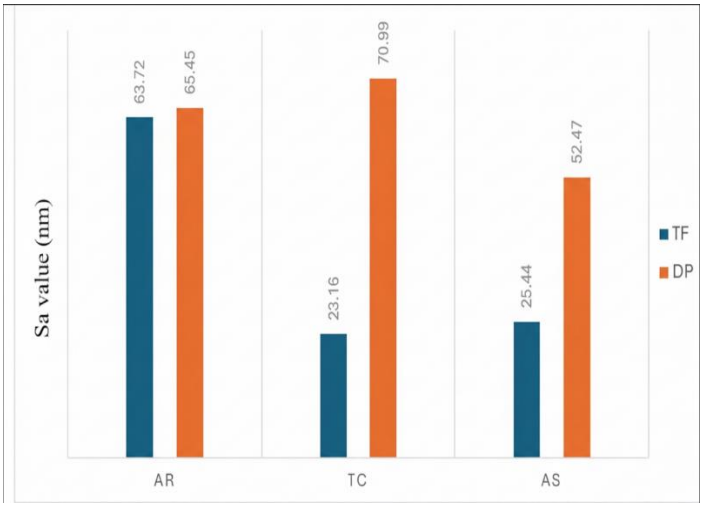


Figure 4: Bar chart comparing mean Sa values (nm) between TF and DP aligners after AR, TC, and AS conditions.
Mean ± SD values: TF/AR 63.72 ± 19.35 nm; DP/AR 65.45 ± 9.93 nm; TF/TC 23.16 ± 13.15 nm; DP/TC 70.99 ± 18.12 nm; TF/AS 25.44 ± 23.15 nm; DP/AS 52.47 ± 5.51 nm.
Sa: arithmetic mean height
TF: thermoformed
DP: directly printed
AR: as-received
AS: artificial saliva
TC: thermocycling

Table 1: Means, standard deviations, and ranges of Sa values for TF and DP groups.							
Condition	N	TF (Mean±SD)	TF (Min–Max)	DP (Mean±SD)	DP (Min–Max)	t	P-value
TC	4	23.16±13.15[a]	15.07–42.79	70.99±18.12	60.66–98.03	-4.273	0.005
AS	4	25.44±23.15[a]	11.15–59.94	52.47±5.51	44.29–55.85	-2.272	0.063
AR	4	63.72±19.35[b]	47.00–90.44	65.45±9.93	53.77–74.65	-0.159	0.879
Within-material ANOVA P-value		0.02		0.14			

P > 0.05=Non-significant, P < 0.05=significant
TF: thermoformed aligners
DP: directly 3D-printed aligners;
TC: thermocycling
AS: artificial saliva immersion
AR: as-received
N: number of specimens per group
SD: standard deviation
t: independent-samples t-test statistic

Discussion

This in vitro study compared the optical and nanoscale surface responses of thermoformed (CA® Pro+) and directly 3D-printed (Tera Harz TC-85 DAC) aligners under three conditions: as-received, after 500 thermocycles, and after 14 days of artificial saliva immersion. Between-material differences after thermocycling were significant for both T% and Sa, and T% differed after saliva immersion. Within each material, TC and AS did not differ significantly. Given the small sample size and the independent-group design, these findings are exploratory.

Light transmittance

Baseline T% was higher for thermoformed aligners than for printed aligners, but the difference was not significant. After thermocycling, T% fell in both materials, more markedly for printed aligners. Transmittance results are consistent with previous spectrophotometric comparisons, which showed that printed and thermoformed clear aligner materials differ in transparency and that this depends on the material and fabrication route (5). Similar differences in optical properties between thermoplastic and printed resin appliances have been reported (3,14), and direct printing of clear aligners has been reviewed with attention to its current limitations in transparency (13). These optical differences may reflect differences in material structure. The thermoformed material is a multilayer thermoplastic polyurethane system, and the printed resin is a cross-linked polymer network. Material-based differences in color and chemical stability between printed and thermoformed polyurethane aligners have been described (12), which is consistent with our observations. However, the material structure was not characterized here, so this explanation remains a hypothesis. Thermocycling has been reported to induce hydrolytic and thermal changes in thermoplastic retainer materials (9), which could increase light scattering, and residual monomer leaching from printed resins under thermal stress has been suggested as a possible contributor to aging (10). These mechanisms were not measured and require direct testing. A key confounder is specimen thickness. The printed aligners were designed at 0.5 mm and the thermoformed sheets were 0.75 mm before thermoforming. Because transmittance depends on thickness, the T% differences cannot be attributed to material alone, and post-fabrication thickness should be measured in future work.

Surface roughness

All mean Sa values were below 500 nm. After thermocycling, Sa was lower for thermoformed than for printed aligners, and this was the only

significant between-material roughness difference. Sa was not significantly different between materials after saliva immersion or in the as-received condition. Within-material changes were not significant for either material. Therefore, the data do not establish that either material became rougher with aging, and the observed trends should not be interpreted as deterioration.

Similar in vivo and in vitro studies have reported aging-related roughness changes in clear aligners, with differences between materials (7,11), but the direction and magnitude of these changes vary with the study design and the exposure. In the present study, the 2 × 2 µm scan area on one tooth position limits how far the results can be extended to whole-aligner roughness or to plaque retention. The 500 nm reference value used here should be regarded as a descriptive benchmark rather than an established clinical threshold.

Clinical relevance and interpretation

Under this short-term, sequential protocol, the aging conditions produced a significant between-material difference after thermocycling for both T% and Sa, favoring thermoformed aligners. The evidence supports a material-related difference under thermal aging rather than a clear degradation trend for either material. Whether 500 cycles correspond to a given period of clinical use has not been established, and patient-reported outcomes were not assessed. Future studies with larger samples, matched thickness, and separately tested aging conditions are needed to confirm these findings and to determine their clinical relevance.

Limitations

This study has several limitations. First, the sample size was small (n = 4 per material per condition), which gives limited statistical power, makes normality and variance tests unreliable, and means that non-significant results should not be interpreted as evidence of no effect. Second, each specimen was exposed to only one condition, so changes within each material were compared between independent groups rather than measured on the same specimens; the AR-to-TC and AR-to-AS changes were not tested statistically. Third, the two materials differed in nominal thickness (0.5 mm for DP vs. 0.75 mm before thermoforming), and the actual post-fabrication thickness of the specimens was not measured. Because light transmittance depends on thickness, the T% differences cannot be attributed to material alone. Fourth, the materials also differed in fabrication route (direct MSLA printing at 45° and 50 µm layers vs. pressure thermoforming), so the material effect is confounded with manufacturing parameters, and the findings should not be generalized to other printing technologies or parameters. Fifth, surface roughness was measured from a single 2 × 2 µm scan per specimen on the facial surface of one tooth position, which is a small area and may not represent the whole aligner surface; the Sa values may therefore vary more than the reported SDs suggest. Sixth, the material composition was not characterized, so the proposed mechanisms (hydrolysis, microcrack formation, monomer leaching) remain hypotheses. Seventh, the aging protocols (500 thermocycles and 14 days of immersion) simulate, but do not reproduce, intraoral conditions, and their relationship to clinical service life has not been established. Finally, the 500 nm Sa threshold used as a reference point lacks a firm clinical basis, and the study did not assess plaque accumulation, patient comfort, or patient acceptance.

Conclusions

Within the limitations of this exploratory in vitro study (n = 4 per group), thermocycling was associated with a significant between-material difference in both light transmittance (T%) and surface roughness (Sa), with thermoformed CA® Pro+ aligners showing higher T% and lower Sa than directly printed Tera Harz TC-85 DAC aligners. Saliva immersion was associated with a significant between-material difference in T% only. Within each material, T% and Sa did not differ significantly between the thermocycling and artificial saliva conditions, and no significant change in Sa was demonstrated for either material. Because the between-material comparisons were made on specimens with different nominal thicknesses (0.5 mm vs. 0.75 mm), the optical differences cannot be attributed to material alone. These findings should be considered hypothesis-generating and require confirmation in larger studies with matched thickness, separate testing of each aging condition, and chemical characterization of the materials.

Acknowledgments
None

Conflict of Interests
The authors declare no conflict of interest.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding
None.

Authors' Contributions

Ibrahim Yawoz Abdulkarim: conceptualization, methodology, investigation, data curation, formal analysis, visualization, and writing of the original draft.

Sara Al-Mashhadany: supervision, methodology, validation, and critical review and editing of the manuscript.

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