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Original Article

Comparative microscopic analysis of plastic dispersion from 3D-printed and thermoformed orthodontic aligners

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Abstract

Aim: To compare directly printed aligners (DPA) and thermoformed aligners (TFA), evaluating the potential release and dispersion of microplastic (MP) and nanoplastic (NP) particles under simulated oral conditions.

Materials and methods: DPA samples (Graphy Tera Harz TC-85-DAC resin) and TFA samples (Invisalign® SmartTrack) were subjected to rubs in an ultrapure water bath. The liquid was collected post-friction and analyzed for MPs and NPs using various techniques: optical microscopy (OM), transmission electron microscopy (TEM), and atomic force microscopy (AFM). Also, plastic residues were quantified by weighing after drying within a laminar flow hood. Microscopic image analyses comprised the quantification of the average size of MPs and NPs, their concentration by TEM, and the roughness analysis by AFM.

Results: The masses of MPs and NPs separated after rubbing were 0.001 g/200 µl and 0.004 g/200 µl for TFA and DPA samples, respectively. TEM analysis confirmed that DPA samples had larger (203.08 ± 2651.65 µm²) and more numerous particles compared to TFA (0.23 ± 27.53 µm²), even though it was not possible to distinguish the MPs and NPs due to clustering of the plastic residuals. AFM analysis indicated a bigger root mean square grain size for TFA than DPA; similarly, the mean roughness was lesser in the DPA sample than TFA one.

Conclusions: DPA generated larger and more numerous plastic particles compared to TFA, though grain-size characterization was challenging due to particle aggregation. This suggests that the manufacturing process and materials used in DPA could impact the creation of MPs and NPs during simulated mastication, highlighting a potential area for process optimization.

Keywords: direct printed aligners; microscopy analysis; microsplastics; nanoplastics; thermoformed aligners

Introduction

Over the years, technological advancements have opened the field of orthodontics to more innovative methods. Digital breakthroughs have transformed diagnosis, treatment approaches, and the design of new orthodontic devices [1]. One of the primary outcomes of digital advancements is the development of clear aligners, which provide a more discreet alternative to traditional braces [2]. The demand for invisible orthodontic treatment has increased, making clear aligners a standard option in orthodontic practice, especially in adult patients [3].

Direct printed aligners (DPA), using 3D-printing technology, represent a cutting-edge advancement in orthodontics [4]. Indeed, in contrast to traditional thermo-formed aligners (TFA), DPA is produced directly from digital scans of patients' teeth and enables the clinician to choose different thicknesses, surface textures, and shapes that enhance the device's biomechanics, minimizing or eliminating the need for attachments [5]. Despite the numerous advantages, the potential toxicity of 3D-printed devices, mainly when they are in constant contact with oral tissues or ingested, should not be overlooked [6]. Thermoplastic materials used by TFA manufacturers include polyethylene terephthalate, polypropylene, polycarbonate, and polyurethanes [7].

All these plastics can be subjected to various environmental and mechanical factors which degrade them into smaller fragments (100–1000 nm), referred to as secondary microplastics (MPs) [8]. Furthermore, factors such as oral temperature variations and exposure to many substances among foods and beverages, as well as intraoral aging, can transform MPs into nanoplastics (NPs) characterized by smaller dimensions than MPs (1–100 nm) [9]. Due to their tiny size, NPSs may easily permeate biological membranes, potentially entering inside cells and tissues or even crossing the blood-brain barrier; however, the potential risks of NPs to human health are still under scientific consideration [10, 11].

DPA is an emerging topic in orthodontics, and its biological effects on human tissues are under review, especially regarding their cytotoxicity [12]. The resin commonly used for DPA is a photopolymer with a polyester-urethane polymer structure [13]. However, rigorous testing is usually conducted to ensure the safety and the biocompatibility of materials according to

regulatory standards, the potential release of MPs and NPs by polymerized orthodontic resins and the subsequent risks to human health remain unknown. Nevertheless, it's important to note that while potential risks are demonstrated, health impact remains subject to ongoing research. Therefore, direct evidence of the effects of MPs and NPs on human health still needs to be expanded to *in-vitro* studies [14].

Therefore, the present study aimed to compare DPA and TFA regarding the potential release and dispersion of MPs and NPs particles under simulated oral conditions. The study incorporated a mechanical stress representative of biting and chewing forces to understand the long-term safety implications of using these orthodontic devices.

Materials and methods

DPA samples consisted of two rectangular foils made from Graphy Tera Harz TC-85 DAC resin (Graphy Inc., Seoul, South Korea), each measuring 25 × 5 × 0.5 mm. These samples were manufactured using a SprintRay PRO 95 3D printer (SprinRay Europe GmbH, Weiterstadt, Germany) following the manufacturer's protocol. To ensure complete polymerization, post-processing was conducted using a Tera Harz Cure THC2 curing device (Graphy Inc., Seoul, South Korea).

A flat rectangular TFA sample measuring 5 × 25 mm of Invisalign® SmartTrack™ aligners (Align Technology Inc., Tempe, AZ, USA), was also prepared for comparative analysis. An STL arch's file was digitally modified to redesign the posterior teeth into a uniform flat surface measuring 8 × 30 mm, enabling the thermoforming of a flat aligner section. This aligner was subsequently cut into 5 × 25 mm samples, and the thickness was measured across several areas of the surface, yielding a mean thickness of 0.57 mm. The reduced thickness, compared to the original SmartTrack™ material's nominal value (0.75 mm), was likely due to the thermoforming process, where stretching of the material to conform to the flat design leads to a slight reduction in thickness. For this study, the outer surface of the aligners was used.

A controlled friction process to simulate mechanical stress representative of rubbing forces was carried out. The samples were placed in Petri dishes cleaned with piranha etch for 5 min (a mixture of H_2SO_4 and H_2O_2 to remove organic residues and ensure surface cleanliness). Each sample was mechanically subjected to friction against another identical sample 120 cycles under constant pressure within a Milli-Q ultrapure water bath (Merck-Millipore, Burlington, MA, USA). To simulate the mechanical wear, an Ender Three Pro (Creality, Shenzhen, China) 3D printer was modified to function as a controlled friction testing system. Also, a custom 3D-printed support was designed and fabricated to securely hold the specimen in place during the abrasion process. A calibrated spring was positioned between the specimen holder and the Petri dish to apply a controlled and reproducible normal force of 1 kg. The applied force was measured and adjusted using a dynamometer, ensuring consistency across all samples. A custom G-code was developed to control the rubbing motion with precisely defined parameters, including a constant speed and uniform stroke length, to ensure reproducibility of the wear process. To achieve a uniform abrasion pattern, the motion was programmed in a figure-eight trajectory, ensuring even distribution of mechanical stress across the specimen surface.

Following the friction process, the bath was sonicated for 15 min at 37°C to ensure the detachment of MP and NP particles from the samples. This friction and sonication processes was designed to simulate the mechanical stress experienced during mastication. All the procedure was performed within a laminar flow hood to ensure a controlled environment and minimize contamination risk.

After this step, the resulting liquid was collected using a precision micro-doser after shaking the bath. The collected liquid was subsequently allocated for various analytical procedures:

- microplastics (>10 µm): 50 µl placed on standard optical microscopy (OM) glass side;
- sub-microplastics (40 nm–10 µm): 20 µl deposited on transmission electron microscopy (TEM) grid coated by formvar resin, using a 300 mesh Au grid (2SPI, West Chester, PA, USA); the residual particles contained in these samples were also subsequently analyzed in terms of size and concentration;
- nanoplastics (<40 nm): 50 µl placed on muscovite mica disks for atomic force microscopy (AFM); the samples were allowed to air dry for 2 h under the laminar flow hood to eliminate any residual water. Also, two mica disks with 200 µl of liquid for the sample were weighed by Gram Core RTI analytical balance (Gram group, Barcelona, Spain) before and after drying to quantify the residual MPs and NPs weight with an accuracy of 0.0001 g.

OM was conducted using a Nikon Eclipse E600 microscope (Nikon Instruments Inc., Melville, NY, USA) under bright-field illumination at 4x magnification, with images captured via a Polaroid DMC digital camera (Polaroid, Waltham, MA, USA). Ten slides were prepared for each sample type, and each was analyzed five times to ensure the reproducibility of the results. TEM analysis was conducted using a JEOL JEM1010 EX transmission electron microscope (JEOL, Tokyo, Japan) at 80kV accelerating voltage and a beam current of 56 µA for high-resolution imaging. Images were captured using the Morada G3 TEM digital camera system (Olympus, Tokyo, Japan). TEM grids allowed for calculating plastic particles due to the contrast between MPs and NPs clusters and the non-contaminated grid surface.

AFM analysis was conducted using a Digital Instruments Nanoscope IIIa (Santa Barbara, CA, USA), with a scan size of 3 × 3 µm, a resolution of 512 lines per frame, and a scan rate of 1.968Hz. The surface characteristics of the samples were examined using Gwyddion v.2.61 software (Czech Metrology Institute, Brno, Czech Republic). AFM was operated in tapping mode to prevent damage to the samples. The Z-axis sensitivity was calibrated to 8.40 nm/V, and piezo calibration was set at 440 V. Imaging was conducted in tapping mode using an amplitude feedback system with proportional and integral gains of 100 each. The drive amplitude, frequency, and phase were 75 mV, 259.17 kHz, and 117.66°, respectively.

The captured images from OM and TEM were analyzed using Fiji software (ImageJ). Images were calibrated, converted to 8-bit grayscale, segmented, and subjected to particle analysis to quantify the size and distribution of MPs and NPs. For TEM, particle sizes were expressed in pixel units, and the percentage of the total microscopic field covered by particles was calculated.

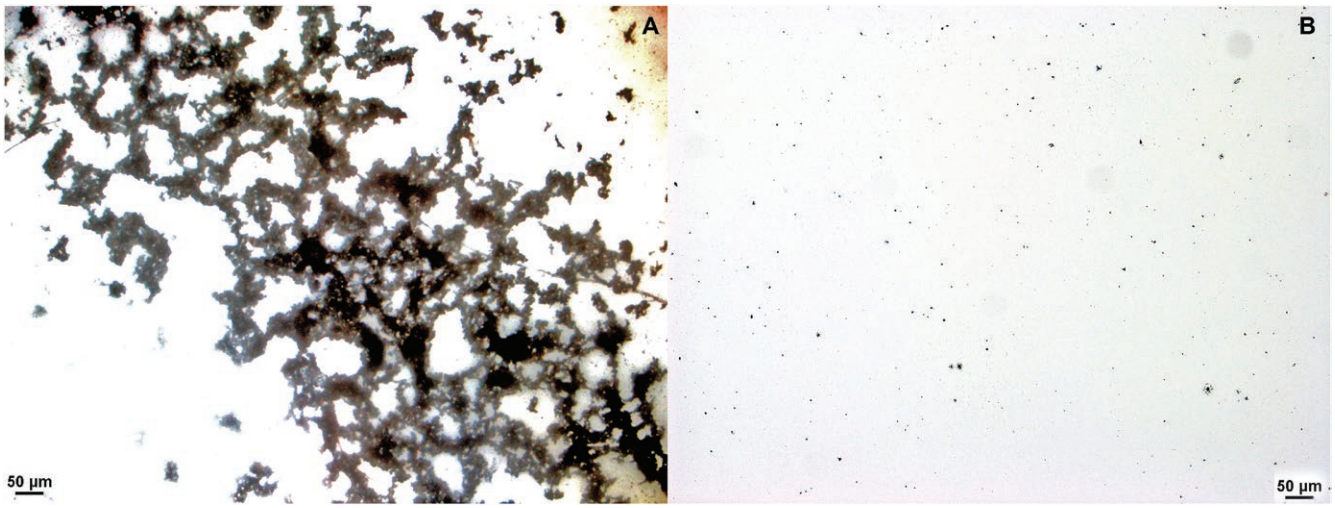

Figure 1. Optical microscopy images at 4x magnification comparing DPA (A) and TFA (B) samples.

AFM data were processed using Gwyddion software to calculate surface roughness parameters, including root mean square roughness (Sq) and mean roughness (Sa). These parameters were used to compare the surface characteristics of the DPA and TFA samples. Five measurements of Sq and Sa were taken for each sample type, including the blank mica disk used as a control to set the instrument parameters.

All statistical analyses were conducted using SPSS v.26 (IBM Corp., Armonk, NY, USA). The selection of statistical methods was based on an initial evaluation of data distribution and variance homogeneity to ensure the appropriateness of parametric testing. Two groups were analyzed, DPA and TFA, each consisting of ten samples. To enhance data reliability and reproducibility, five replicates were taken per measurement for each sample.

The normality of continuous variables was assessed using the Shapiro-Wilk test, with p-values greater than 0.05 indicating normal distribution. Mean values and standard deviations (SDs) were calculated for all measured variables to summarize the data comprehensively.

For inferential statistical testing, since data were normally distributed, independent samples t-tests were used to compare DPA and TFA groups when normality assumptions were met. To compare multiple repeated measurements within the same group, a repeated-measures ANOVA was conducted. The significance threshold was set at $P < .05$ for all tests.

Results

The weight loss of DPA and TFA samples was measured before and after the simulated mechanical stress to evaluate the potential release of MP particles from the aligners. This was measured by weighing the mica disks alone and then weighing the same disks after the eluent suspension of plastics (200 µl) was applied and dried. The difference in weight reflects the mass of MPs released during the mechanical stress, indirectly measuring the quantity of particles generated. The DPA sample exhibited a consistent mean weight loss of 0.004 ± 0.0001 g. In contrast, the TFA aligners showed a smaller and consistent mean weight loss of 0.001 g with no observed variability. A two-sample t-test confirmed that this difference was statistically significant ($P < .0001$), indicating that the DPA samples released significantly more MP and NP particles than the TFA samples under laboratory conditions.

OM was employed to compare the distribution of MP particles released from both aligners (Fig. 1). Ten slides were prepared for each sample type and analyzed five times. The DPA slides showed significantly larger MP particles than TFA samples. Additionally, the percentage of the microscopic field covered by MPs and NPs particles was substantially higher for DPA samples at 32.34%, compared to 1.07% for the TFA samples. Statistical analysis using a t-test confirmed significant differences in average particle size and percentage area covered between the two aligner types ($P < .0001$). ANOVA showed no statistically significant intra-group variations, indicating consistency within each group ($P > .05$).

TEM was utilized to assess the size of NP particles released from the aligners further (Fig. 2). Quantitative analysis indicated that particles from DPA tended to form aggregated clusters. Quantitatively, the average particle size in DPA samples was approximately 1000 times larger than that of TFA samples. DPA samples displayed an average grain size of 216.23 ± 44.5 µm², while TFA particles averaged 0.24 ± 0.09 µm². Furthermore, the total amount of plastic residuals found on a single grid was 283.50 µm² for DPA and 16.49 µm² for TFA. When comparing the total areas examined – 8650 µm² for DPA and 6473 µm² for TFA—the proportion of the area covered by plastic particles was 3.3% and 0.3%, respectively. This indicates that the concentration of MPs released from DPA aligners was approximately 10 times greater than that of TFA aligners. These findings were corroborated by a statistical analysis conducted on 10 grids per sample type, with measurements repeated five times. A two-sample t-test confirmed that the differences between the two groups were statistically significant ($P < .0001$). No statistically significant intra-group variations were detected by repeated ANOVA ($P > .05$), indicating consistent measurements within each group. The higher variance observed in DPA samples compared to TFA samples suggested that the 3D-printed aligners release larger particles and exhibit greater particle size and quantity variability.

AFM was employed assess the presence of NPs particles (Fig. 3). The primary metrics analyzed were Sq and Sa. Each sample type was measured five times, and average values with

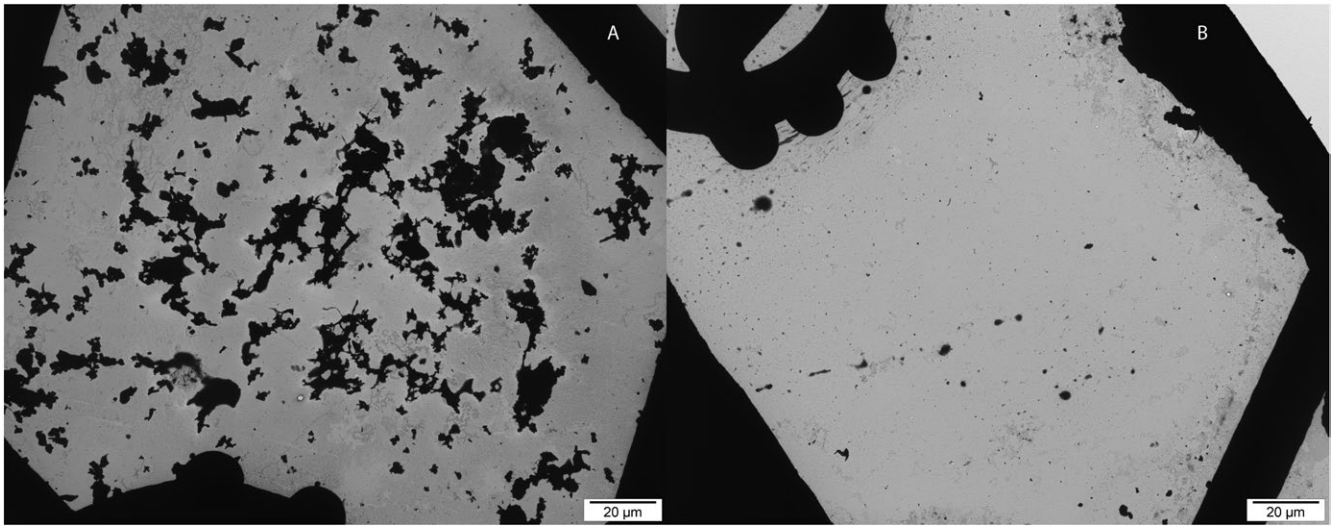

Figure 2. TEM images at low magnification, comparing DPA (A) and TFA (B) samples.

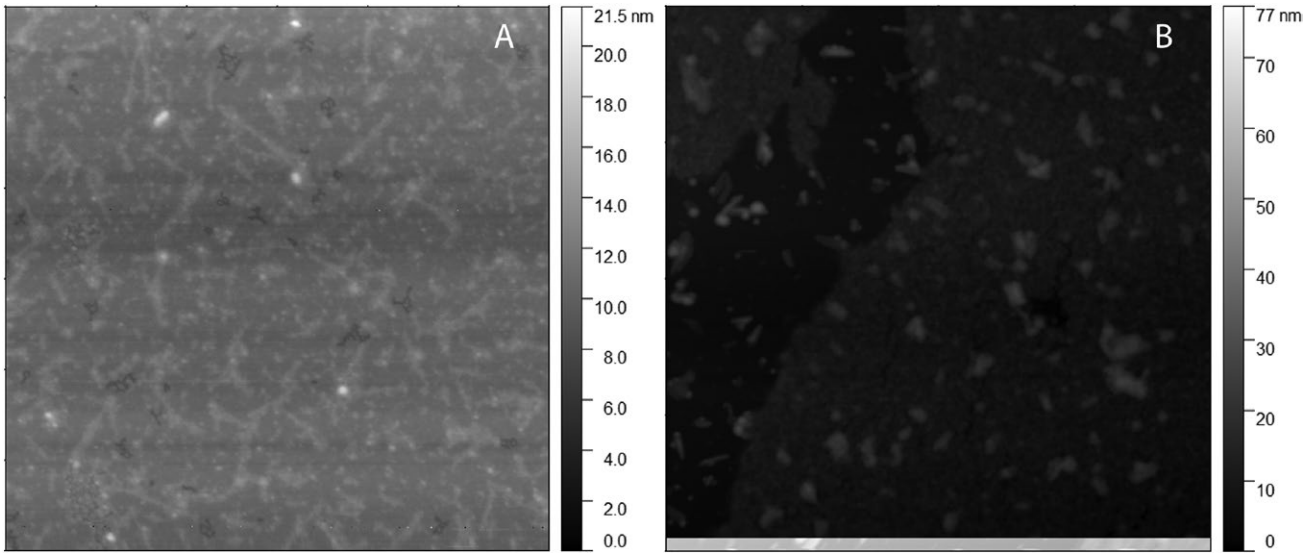

Figure 3. AFM images, comparing DPA (A) and TFA (B) samples.

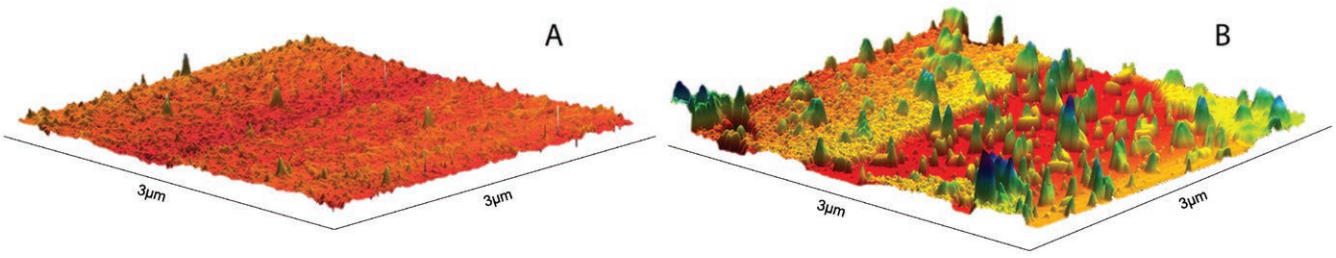

Figure 4. 3D surface reconstruction of the height map of DPA (A) and TFA (B) observed under 3 × 3µm AFM surface.

standard deviations were calculated. TFA samples demonstrated higher roughness, with an Sq of 160.08 ± 68.49 nm and a Sa of 102.61 ± 21.45 nm. DPA samples, in contrast, exhibited significantly lower roughness, with a Sq of 5.48 ± 1.38 nm and a Sa of 3.28 ± 0.85 nm, indicating a much smoother surface (Fig. 4). The t-test performed on the five replicates for each sample type showed no statistically significant intra-group differences ($P < .001$), confirming consistency within each group. Inter-group inferential statistics showed significant differences between TFA and DPA sample for Sa ($P = .025$) but not for Sq ($P = .357$). AFM characterization of the mica disks confirmed the planarity of the surface and ensured no contamination from external sources. The maximum peak heights measured were 36.55 nm for

Table 1. Results of descriptive statistical analysis for weight loss, OM, TEM, and AFM data, including mean values and standard deviations (SDs). Table summarizes the normality test results (Shapiro-Wilk test) and it displays the inferential statistics outcomes (p-values), showing significant differences between DPA and TFA samples.

	TFA		Shapiro-Wilk test	DPA		Shapiro-Wilk test	P-value
	Mean	SD		Mean	SD		
Weight loss (g)	0.004	0.001	0.966	0.001	0	0.935	<.0001
OM—Area covered (%)	1.07	0.33	0.265	32.34	3.82	0.201	<.0001
TEM –Grain size (μm²)	0.24	0.09	0.942	216.23	44.5	0.849	<.0001
AFM—Sq (nm)	160.08	68.49	0.979	5.48	1.38	0.958	.357
AFM—Sa (nm)	102.61	21.45	0.940	3.28	0.85	0.861	.025

TFA and 144.75 nm for DPA, demonstrating that this last exhibited larger peaks despite having a smoother overall surface. Moreover, the blank mica control sample recorded intermediate roughness values, with a Sa of 97.87 nm, a Sq of 125.36 nm, and a maximum peak height of 9.83 nm. This confirmed the suitability of the mica substrate as a control in the analysis.

Table 1 summarizes the descriptive statistical analysis of weight loss, OM, TEM, and AFM data, providing mean values, standard deviations (SDs), and inferential statistical outcomes. In conclusion, the results indicated significant differences between TFA and DPA in terms of weight loss, OM, and TEM measurements ($p < .0001$), while AFM roughness parameters (Sq and Sa) showed mixed statistical significance.

Discussion

The widespread popularity of clear aligners has led different brands to launch various alternatives of these aesthetic and comfortable orthodontic devices on the market [1–3]. All of them are made from thermoplastic polymers, and among the different types of materials, polyurethane, polyester, and polyethylene terephthalate are the most used [15].

Only a few studies have reported that these materials exhibit unstable behavior over time, particularly when subjected to mechanical stress or the oral environment [8, 16, 17]. Indeed, the daily wearing of clear aligners produces constant frictional contact between the upper and lower occlusal surface, inevitably leaving plastic fragments in the oral cavity. It was already demonstrated that the percentage of these plastic residuals is directly correlated to the hour per day, and the period aligners are recommended to be worn [8].

Nowadays, several studies have been performed to evaluate the stability of these materials in terms of mechanical proprieties, aging, colorimetric alterations, and chemical changes which can compromise force capacity and treatment efficacy. It was proven that different polymerization procedures may affect the *in-vitro* cytotoxicity of 3D-print resins. However, no information has been given about the size and the weight of plastic fragments left after any mechanical stress once they have been light-cured [18]. A further *in-vitro* study, without any focus on plastic residuals, had already confirmed how polymerization and washing procedures affected the cytotoxicity instead [6].

However, only a small number of studies considered a qualitative and quantitative evaluation of aligners' structural changes and, particularly, the differences in behavior between DPA and TFA [19, 20]. Due to the lack of knowledge on this topic, the present study was conducted through OM, TEM, and AFM to highlight the macro and micro characteristics of plastic fragments, both MPs and NPs, produced by orthodontic DPA and TFA.

Weight

The differences observed between DPA and TFA samples are quite revealing in assessing the impact of the potential for systemic MP detachment. DPA samples exhibited a fourfold increase in the weight of MPs compared to TFA, indicating a substantially higher level of contamination. Specifically, the weight gain due to MPs for DPA was measured at 0.004 g, while TFA samples showed a more modest increase of 0.001 g. What's particularly noteworthy was the uniformity of MP adhesion across samples within each material category. Both DPA and TFA samples demonstrated consistent weight changes, implying a predictable pattern of MP adhesion on their respective surfaces.

While the absolute weight changes due to MP accumulation were relatively small in numerical terms, the implications of this finding are far from insignificant. The slight weight gain was meaningful given the measurement scale at which these measurements were taken, highlighting a genuine concern over material contamination.

Taking the initial weight measurement as a baseline, representing the stub with mica devoid of any sample, the subsequent increase in weight can be confidently ascribed to the MPs that have adhered to the surface of each stub only. This indirect comparison clearly indicates the presence and quantifiable impact of MP contamination on the materials in question.

It is important to note that these gravimetric changes reported in this study were based on the weight gain measured on mica disks after the application and drying of 200 μl of eluent collected from the friction bath. These measurements do not directly represent the weight loss of the original specimens but rather quantify the detached particles in the eluent.

Moreover, this negligible contamination must be correlated with the dimension of the analyzed samples and the time under mechanical stress, both significantly and clinically underestimated than the real dimension of an aligner worn on teeth and undergoing functional and nonfunctional chewing or grinding.

Optical microscopy

From this comparison, we can infer that DPA samples have a significantly higher presence of MPs in terms of the size of individual particles and their distribution across the sample

area. This could be due to various factors, including differences in material composition, the 3D-printing process used for printed aligners, or the efficacy of the post-manufacturing processes, i.e. cleaning and finishing. The substantial area occupied by MPs in DPA samples could have implications for the quality and performance of the product.

OM is the most commonly reported method for initial visual and physical characterization of MPs, probably because it is a robust and inexpensive system. Still, this technique cannot provide a clear distinction between MPs and NPs as well as recognizing single particles or clusters [21]. As the matter of fact, the real size and distribution of the particles were challenging to determine due to the tendency of smaller plastic fragments to aggregate, making it impossible to identify individual particles or their precise distribution accurately.

Transmission electron microscopy

Through the lens of the TEM images, a substantial divergence in the purity of the two materials was observed. The TEM images revealed that DPA material harbors an overall accumulation of significantly higher debris than TFA material. To quantify, DPA samples were burdened with a total debris sum that surpasses TFA by an order of magnitude, a factor that may implicate the material's surface chemistry or physical texture in its affinity for particle retention.

Diving deeper into the average debris size, the disparity grows starker, with DPA samples presenting with particles that are, on average, hundreds of times larger than those seen on TFA samples. In the realm of TEM, where even the minutest surface imperfections are laid bare, such a discrepancy highlights a potential issue with aligner printing material's consistency and its interaction with environmental particulates. It is hypothesized that minimal atomic-level forces and the surface tension of these materials can cluster the smallest NP fragments and accumulate them into larger MP clusters, highly variable in dimensions [22]. These findings were observed in ultrapure water, and the clustering behavior might be different in the oral cavity wet by saliva or mucus [23]. A previous study about plastic detachment from TFA carried out by electron microscopy reported the size of secondary debris from 3 nm to 50 nm [7]; nevertheless, these results cannot be compared to the ones from the present study since plastic fragments were isolated after 7 d of mechanical stress in artificial saliva.

The notably higher standard deviation indicated the extensive breadth of debris size distribution on DPA surfaces. This suggests an erratic contamination profile, potentially impacting the material's performance in applications where surface homogeneity is crucial. While the total examined area for DPA samples was greater, this factor did not diminish the relative density of debris on its surface. Regarding debris density per square micrometre, DPA exhibited a markedly higher level than TFA, reinforcing the visual evidence from the TEM images of a significant cleanliness issue.

Atomic force microscopy

First, it is well known that AFM is primarily utilized as a visual proof of the presence and behavior of the particles rather than for direct particle size characterization. Although a simpler approach, gravimetric analysis was considered the primary method for estimating the approximate magnitude of MP production. It is important to note that, consistent with the behavior of MPs and NPs, the particles tend to aggregate, especially during drying.

The analysis commenced with TFA samples, which exhibited significant surface roughness as evidenced by the mean roughness values. This roughness was characterized by considerable variability, as demonstrated by the higher standard deviations across the measured parameters. Such variability may imply a non-uniform surface modification process or intrinsic material characteristics leading to a varied topography reminiscent of a natural landscape sculpted by different environmental factors. Conversely, DPA samples delineated a contrasting narrative, with a generally smoother surface profile denoted by lower mean roughness values. The mica-adhered stubs, used as a control sample, were intended to provide a baseline of negligible surface roughness, affirming the AFM's capacity for accurate measurement in a clean environment. Paradoxically, these controls displayed an unexpected level of variability, with a notable standard deviation in the average roughness. This finding necessitates a critical assessment of the assumed flatness of mica surfaces and the potential influence of substrate irregularities on the AFM analysis.

Despite the different purposes, two previous *in-vivo* studies reported that 3D-printed aligners suffered a greater alteration in surface roughness and porosity compared to TFA [24, 25]; these changes might be related to a mechanical surface alteration as well as MPs and NPs production and detachment.

As previously mentioned, due to surface tension effects, residues were more concentrated at the center of the substrate and less at the edges, creating bigger clusters that do not reflect the real dimension of the single MP and NP particles. These factors highlighted the challenges of accurately quantifying and characterizing individual particle sizes despite under these controlled conditions. In fact, several analytical techniques are available for MP/MP characterization, including dynamic light scattering and nanoparticle tracking analysis, which can provide insights into size distribution. However, these techniques primarily function in liquid suspensions, where spontaneous aggregation of plastic residuals can lead to misleading size estimations. The tendency of NPs to cluster due to surface tension, electrostatic interactions, and solvent effects limits the ability to distinguish individual particles, especially during drying and sample preparation.

Limitations

This study has several limitations regarding the translation of the present findings from laboratory to clinical scenarios. Firstly, the simulation of mastication may not perfectly replicate the complex dynamics of actual human chewing, potentially affecting the generation and behavior of MPs. As the matter of fact, this study utilized flat samples to simulate mechanical stress. Scrubbing flat samples primarily can expose the outer layer of multilayer materials, whereas using non-flat samples (covering cusps and fossae) under intraoral conditions could increase exposure of the inner material, potentially altering the results regarding MP release. Moreover, abrasion from aligner materials in the oral environment involves not only occlusal surfaces but also fitting surfaces, particularly at composite resin attachment interfaces, which were not replicated in our study, as well as dental morphology. Additionally, the absence of saliva, which contains microparticles like calcium and phosphate, may underestimate friction forces and the dispersion of plastic particles.

Finally, while the study provided quantitative data on the size and coverage of MPs, it did not assess the chemical composition of these particles and it did not analyze any cumulative dose during time; understanding the composition and the MPs and NPs accumulation is crucial for evaluating potential toxicity and its impact on human health, necessitating further research.

As a result, while the study provides important preliminary findings, all these limitations highlighted the need for further *in vivo* studies better to quantify the actual particle release in clinical scenarios.

Conclusions

This investigation focused on generating MPs and NPs from dental aligners, utilizing a simulation of mastication to explore differences between aligners produced via traditional thermoforming method and those crafted using 3D printing technology. Detailed analyses were conducted using OM, TEM, and AFM to assess the size and distribution of MPs and NPs particles. The findings revealed significant variations between the production techniques. DPA produced larger and more numerous plastic particles than TFA under laboratory-simulate conditions; however, grain-size characterization was challenging due to spontaneous aggregation into bigger clusters.

This study was conducted under controlled *in vitro* conditions, which inherently limit the clinical relevance of the findings. Beyond the simplified bite simulation, other factors, such as the use of flat samples, the forces applied, and the absence of dynamic oral conditions (e.g. saliva, temperature fluctuations, and variable forces), further restrict the generalizability of the results, as well as cannot be directly extrapolated to clinical scenarios without further studies.

Despite that, these results can indicate that DPA's manufacturing process, and material choices might influence MPs and NPs' generation during simulated mastication, pointing to a potential area for process optimization.

It is essential to clarify that this study did not explore the effects of these residuals on human health. The implications of plastic ingestion or exposure due to using orthodontic aligners remain beyond the scope of this qualitative and quantitative analysis.

Author contributions

Piero Antonio Zecca (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Investigation [equal], Methodology [equal], Software [equal], Writing—original draft [equal]), Marina Borgese (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Investigation [equal], Methodology [equal], Software [equal], Writing—original draft [equal]), Mario Raspanti (Project administration [equal], Resources [equal], Supervision [equal]), Francesca Zara (Visualization [equal], Writing—review & editing [equal]), Rosamaria Fastuca (Supervision [equal], Writing—review & editing [equal]), Marco Serafin (Conceptualization [equal], Validation [equal], Visualization [equal], Writing—review & editing [equal]), and Alberto Caprioglio (Project administration [equal], Resources [equal], Supervision [equal])

Conflict of interest

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

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Data availability

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

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