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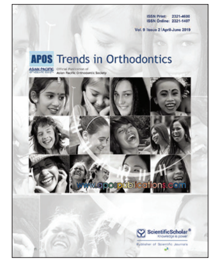

Original Article

Evaluation of microplastic release from orthodontic clear aligner – A spectroscopic study

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ABSTRACT

Objectives: The purpose of this study was to assess how mechanical friction caused microplastics (MPs) to be released from clear aligners in simulated oral environments. The study's objectives were to compare MP release between friction-exposed and control samples, which characterize the MPs using Raman Microspectroscopy (RMS) and Scanning Electron Microscopy (SEM) clear aligners are made of different types of polymers, such as polyethylene terephthalate (PET), polyurethane (PU), or polycarbonate (PC). These polymers are susceptible to a number of mechanical and environmental conditions that can break them down into smaller pieces known as secondary MPs. MPs are synthetic polymer fibers or particles with a diameter ranging from 1 to 5000 μm. Identifying the original components of MPs and their possible effects on the environment and human health is difficult since they can also absorb other contaminants.

Material and Methods: Clear aligners were immersed in artificial saliva and subjected to mechanical friction using a linear reciprocating tribometer for durations of 3 h, 5 h, 7 h, and 7 days. MPs released were isolated and analyzed using RMS to identify their chemical composition and SEM to characterize their size and morphology. Control samples were stored in artificial saliva without friction exposure.

Results: The study found that mechanical friction significantly increased MP release from clear aligners, with particles as small as 5 μm detected. The quantity of MPs released increased with exposure time, reaching the highest levels after 7 days. Control samples without friction showed no detectable MP release, confirming mechanical stress as the primary factor.

Conclusion: Prolonged use of clear aligners results in the release of MPs due to mechanical friction, posing potential risks to oral health and the environment. The findings highlight the need for biocompatible and sustainable materials to minimize microplastic pollution and its associated impacts.

Keywords: Aligners, Endocrine disruptors, Microplastics, Orthodontic appliances

INTRODUCTION

The increasing number of adult and pediatric patients seeking "invisible" orthodontic treatments has spurred the development of more comfortable and esthetically pleasing fixed appliance substitutes.[1] Clear aligners have transformed orthodontic treatment, offering a discre*et al*ternative to traditional braces. The concept of using removable appliances to move teeth dates back to the early 20th century. However, the modern clear aligner system began in 1997 when Align Technology introduced Invisalign, the first commercially successful clear aligner system. Using 3D imaging and computer-aided design/computer-aided manufacturing technology, aligners allowed for the creation of customized, transparent plastic trays that gradually moved teeth into position. This innovation revolutionized orthodontics, providing an esthetic, comfortable, and convenient treatment option for patients.[1,2] Over time, various companies have developed similar systems, advancing clear aligner technology through better materials and more precise digital treatment planning. The company recommends that the patient wear each aligner for seven to fourteen days, up to 22 hours a day.[3] Given the length of time worn, there are concerns about the materials being utilized and how they may degrade inside the oral cavity. Clear aligners are transparent thermoplastic trays that fit over the teeth and apply controlled forces to move them gradually. In 1998, align technology produced the first digitally developed and manufactured removable polyurethane aligners based on the Invisalign system. According to the manufacturer, the patient should wear each aligner for up to 22 h/day for 7–14 days.[3]

Clear aligners are made of different types of polymers, such as polyethylene terephthalate (PET), polyurethane (PU), or polycarbonate (PC). These polymers are susceptible to a number of mechanical and environmental conditions that can break them down into smaller pieces known as secondary microplastics (MPs). MPs are synthetic polymer fibers or particles with a diameter ranging from 1 to 5000 μm.[4] Due to their pervasiveness in a variety of ecosystems, including aquatic environments, soil, and even our air, MPs have emerged as a major global environmental concern. The decomposition of bigger plastic objects, the deterioration of synthetic textiles, and the deliberate addition of MPs to industrial and personal care products are some of the sources of these microplastic particles.[5-7] A lesser-known aspect of MPs is still unknown, despite the fact that extensive research has been done to understand their effects on the environment and human health.[8]

Primary and secondary MPs can be identified. The major source of MPs in dentistry is toothpaste that contains plastic particles smaller than 5 mm. When composite restorations are finished and polished, secondary MPs are created from the resin-based components that break down within the mouth or may occasionally leak out.[9] MPs become "Novel" pollutants in the past 10 years due to their widespread dispersion and toxicity.[10] Microplastic intake by people can be dangerous. An increased risk of neoplasia and chronic inflammation may result from the immune system's incapacity to eradicate artificial particles. [10,11] The size, shape, and chemical makeup of MPs determine their potential toxicity. While bigger particles require active endocytosis, very tiny particles can passively permeate the cell membrane.[11]

The small size, uneven form, and varied composition of MPs might make their identification difficult.[12,13] Identifying the original components of MPs and their possible effects on the environment and human health is difficult since they can also absorb other contaminants. A molecular spectrum, such as infrared (IR) spectroscopy or Raman spectroscopy, is frequently used to detect MPs. These spectra may be used to identify the chemical components of the polymers by comparing them to fingerprint-like molecular structures. Among its benefits over IR are its great lateral resolution, ease of sample preparation, and lack of interference from water.[14] Following surface scanning, hundreds to thousands of spectra can be gathered as a hyperspectral matrix to provide a chemical picture that permits size and form analysis.[15,16] When compared to single-spectrum or single-peak analysis, this type of image analysis detection is preferable since, statistically speaking, the sensitivity or analysis certainty may be increased. This is especially crucial for the study of nanoplastics as, in contrast to MPs, the signal may be further weakened by the smaller size. Raman microscopy (RMS) is a highly reliable technique for the detection of MPS not only morphological features of MP but also chemical composition in terms of polymer matrices and pigments.[17]

A recent study by Quinzi *et al*.[4] aimed to chemically identify the polymeric matrix of orthodontic clear aligners and measure the number and size of MPs released due to mechanical friction, using Raman Microspectroscopy (RMS) and Scanning Electron Microspectroscopy (SEM).[15] However, their study had limitations in exposure duration and mechanical simulation, which our study addresses by extending the wear simulation period and improving friction modeling for more realistic results. The artificial saliva was filtered to evaluate the potential detachment of MPs by clear aligners. The aim of the study is to chemically identify the polymeric matrix of MPs and to measure their number and size. The release of MPs from orthodontic clear aligners raises concerns for both human health and the environment. In the oral cavity, MPs may be ingested or absorbed, potentially causing oxidative stress, inflammation, and cytotoxic effects, while also leaching harmful additives such as bisphenols and phthalates that can disrupt hormonal balance. In addition, MPs can alter the oral microbiome by serving as carriers for bacteria. Environmentally, MPs from aligners can enter wastewater, contributing to water contamination, bioaccumulation in aquatic organisms, and disruption of ecosystems, with potential repercussions for human health through the food chain. Improper disposal further leads to soil and air pollution, as MPs persist in the environment due to their non-biodegradable nature.

MATERIAL AND METHODS

Materials

Materials used for the evaluation include clear aligners (Invisalign), artificial saliva ([ICPA Health products Ltd

ICPA] moist mouth), borosilicate glass Petri dishes, and mechanical friction produced by a linear reciprocating tribiometer in artificial saliva. The temperature of artificial saliva used in studies typically ranges from 37°C (98.6°F), which mimics normal human body temperature, to slightly higher temperatures (e.g., 40°C) in accelerated aging studies. This ensures that experimental conditions closely resemble the oral environment during aligner use.

Sample size calculation

F tests:	ANOVA: Fixed effects, omnibus, and one-way		
Analysis:	A priori: Compute required sample size		
Input:	Effect size f =	2.5	
	A err prob =	0.05	
	Power (1-β err prob) =	0.8	
	Number of groups =	3	
Output:	Non-centrality parameter λ	=	37.5000000
	Critical F =	9.5520945	
	Numerator df =	2	
	Denominator df =	3	

Total sample size = 6 (2 in each group)

Group sample 1=2 Group sample 2=2 Group sample 3=2

Actual power = 0.8626090

The sample size for analysis of variance (ANOVA) was calculated using Cohen's f = 2.5, $\alpha = 0.05$, power = 0.8, and three groups. The non-centrality parameter (λ) was 37.5, with a critical F-value of 9.552. Using G*Power, the total required sample size was 6 (2 per group), achieving an actual power of 0.8626.

Methods

This is an *in vitro* study. To identify the existence of microplastic release brought on by mechanical friction, clear orthodontic aligners were utilized. This study uses four test samples and one group of aligners (Invisalign). A control group will be placed in a Petri dish with artificial saliva and left undisturbed for seven days without any mechanical friction. To detect the release of MPs from clear aligners, one aligner from the first sample will be kept in a linear reciprocating tribiometer for 3 h while an aluminum ball moves linearly to stimulate mechanical friction. In a similar manner, the second, third, and fourth samples of clear aligners will be submerged in artificial saliva (ICPA Wet mouth) and kept in a linear reciprocating tribiometer for 5 h, 7 h, and 5 h a day for 7 days in a row, mimicking the mechanical friction of teeth in a physiological setting.

The control group sample which is kept without any mechanical friction is filtered and analyzed at first. The 1st sample which is kept in a tribiometer is collected in a centrifuge tube after 3 h, the artificial saliva will be filtered; then, filters will be analyzed. After 5 h,7 h, and 7 days, the 2nd, 3rd, and 4th sample is collected in a centrifuge tube, and the filtrate will be analyzed using Raman Microspectroscopy (RMS) and Scanning Electron Microspectroscopy (SEM), respectively, to chemically identify the polymeric matrix and to measure the number and size of the detected MPs.

Calibration details

Equipment used: Linear reciprocating tribometer.

Control conditions: Artificial saliva without mechanical friction for the control group.

Test conditions: Mechanical friction was applied for durations of 3 h, 5 h, 7 h, and 7 days.

Analysis techniques

Raman Microspectroscopy (RMS): For chemical composition analysis.

Scanning electron microscopy (SEM): For size and morphology characterization.

Sample size calculation

Based on ANOVA: Fixed effects, omnibus, and one-way.

Effect size, α error probability = 0.05, Power = 0.8.

Total sample size: 6 (2 samples in each of three groups)

Manufacturer details

Aligners used: Invisalign.

Artificial saliva: ICPA moist mouth.

Material composition: Polymers such as PU, PET, and PC.

RESULTS

In the study, microplastic release was observed from the clear orthodontic aligners (Invisalign) under various conditions of mechanical friction [Figure 1]. The control group, which was the 1st sample kept in artificial saliva without any mechanical friction for 7 days, showed no significant release of MPs. However, the test samples that underwent mechanical friction demonstrated the presence of MPs in the filtrate [Figure 2]. For the 2nd sample, which was exposed to mechanical friction for 3 h, a small but detectable amount of microplastic particles was found. The number of particles increased significantly in the 3rd and 5th samples, which were subjected to 5 and 7 h of mechanical friction, respectively. The sample, which was exposed to mechanical friction for 7 days, showed the highest release of MPs [Figures 3-6]. Raman Microspectroscopy (RMS) and Scanning Electron Microspectroscopy (SEM) were used to analyze the filtrates. RMS confirmed the chemical composition of the MPs as matching the polymeric matrices of the clear aligners, while SEM helped in quantifying the size and morphology of the particles. The artificial filtrate was analyzed for the presence of microplastic under vaccum, which detected the presence of microplastic fibers from clear aligners with the presence of elements such as carbon (C), Oxygen (O_2), silicon (Si), sodium (Na), aluminum (Al), potassium (K), and Zinc (Zn) [Figures 7 and 8] [Graphs 1 and 2] [Tables 1 and 2].

DISCUSSION

The release of consumer products like clear aligners contributes to the growing concern about the role of MPs in global pollution. These tiny particles, ranging from 1 to 5000 µm, are challenging to detect and remove from the environment. The present study extends these concerns to human health, suggesting that chronic exposure to MPs through orthodontic devices may have deleterious effects. While the direct health impact of these MPs is not yet fully understood, studies suggest that they may lead to chronic inflammation, immune system disruption, and even an increased risk of cancer due to the body's inability to expel them.

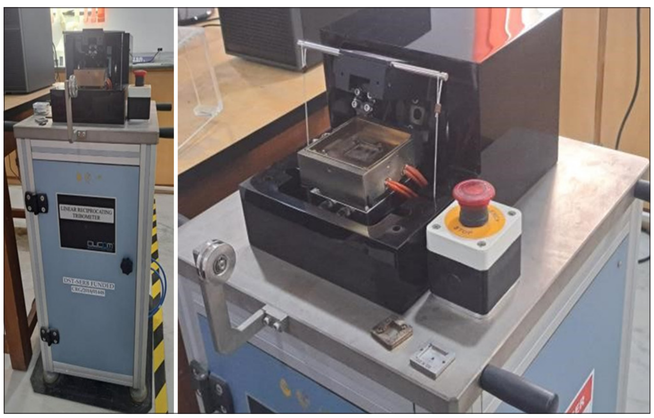


Figure 1: Linear reciprocating tribiometer which simulates mechanical friction.

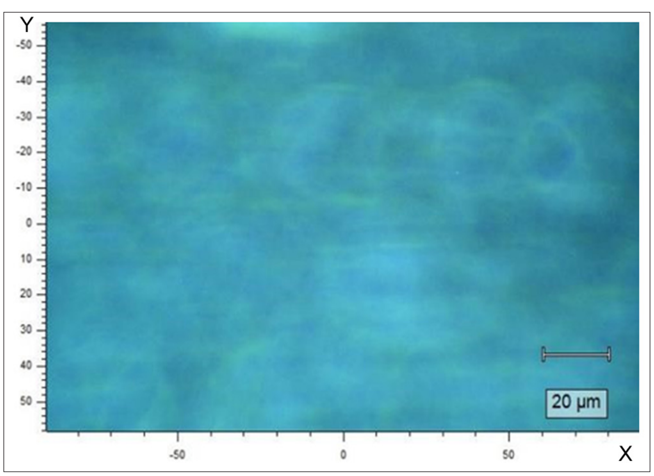


Figure 2: Control sample without any microplastic fibers present due to absence of mechanical friction.

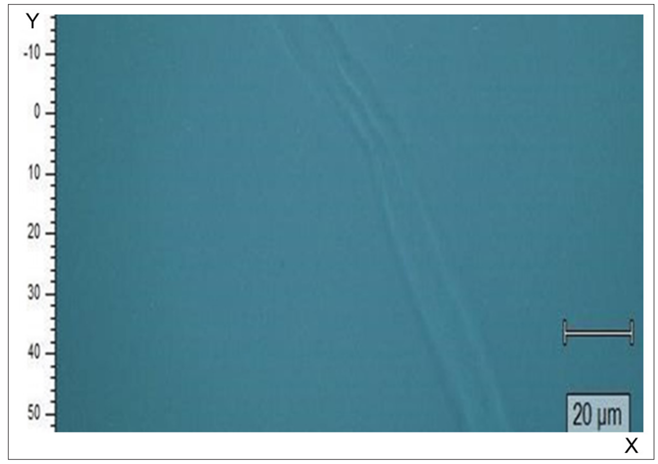


Figure 3: Three hours sample with presence of microplastic fiber.

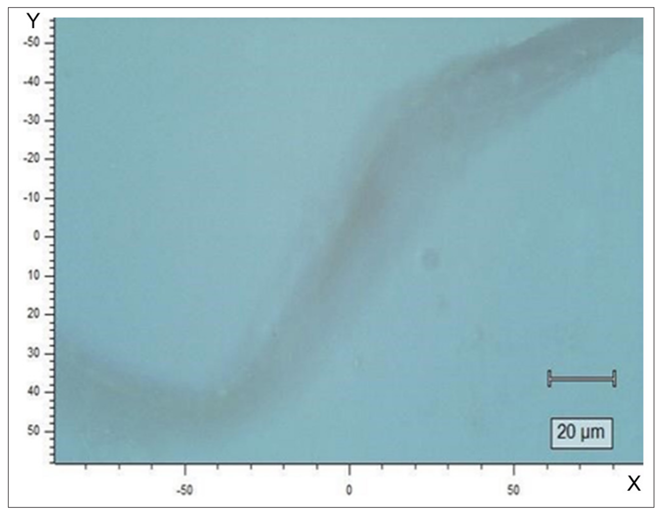


Figure 4: Five hours sample with the presence of microplastic fiber.

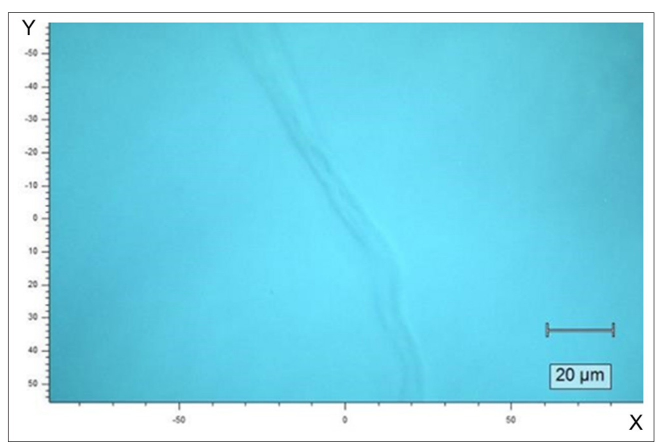


Figure 5: Seven hours sample with the presence of microplastic fiber.

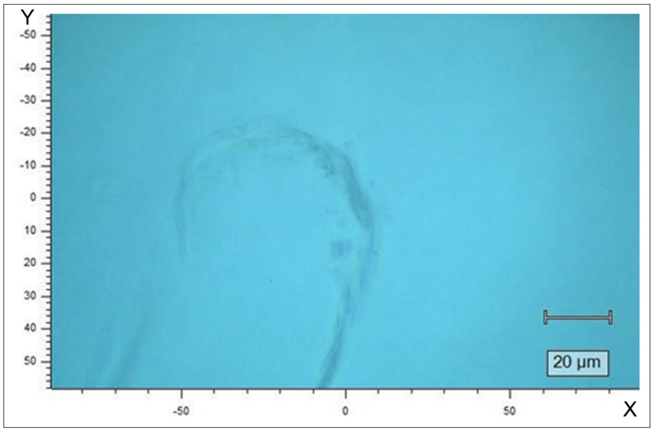


Figure 6: Seven days sample with the presence of microplastic fiber.

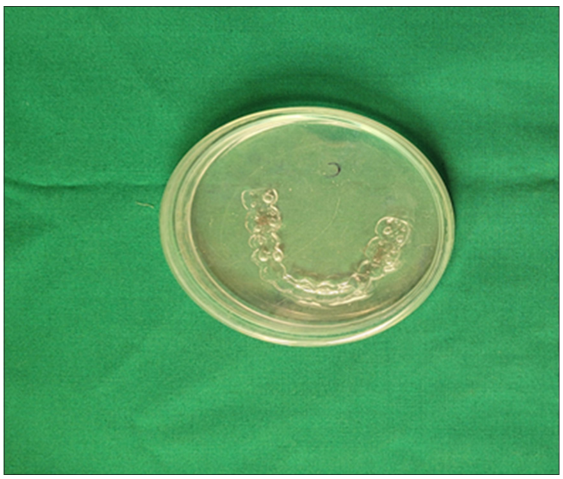


Figure 7: Control sample with absence of mechanical friction.

Table 1: The peak of elements such as carbon and oxygen in SEM analysis of clear aligners.

Map Sum Spectrum	Line Type	Weight %	Weight % Sigma	Atomic %
Element				
C	K series	52.66	0.12	59.70
O	K series	47.34	0.12	40.30
Total		100.00		100.00

C: Carbon, O: Oxygen, SEM: Scanning electron microscopy

Table 2: The presence of elements such as Na, Al, Si, K, and Zn.

Map Sum Spectrum	Line Type	Weight %	Weight % Sigma	Atomic %
Element				
C	K series	54.11	0.15	61.25
O_2	K series	45.24	0.15	38.45
Na	K series	0.35	0.02	0.21
Al	K series	0.08	0.01	0.04
Si	K series	0.01	0.01	0.01
K	K series	0.04	0.01	0.01
Zn	K series	0.17	0.04	0.03
	Total		100.00	

C: Carbon, O: Oxygen, Na: Sodium, Al: Aluminium, Si: Silicone, K: Potassium, Zn: Zinc

The size, shape, and chemical composition of MPs determines their potential toxicity. Smaller particles can passively permeate cell membranes, while larger particles may require active endocytosis. In the case of orthodontic aligners, MPs as small as 5 µm were detected, which raises concerns about their ability to infiltrate biological tissues. Given the increasing body of research on the toxicity of MPs, this study suggests that prolonged exposure to MPs from orthodontic devices should not be overlooked.

The release of MPs in the oral cavity can be influenced by increased friction, particularly from dental appliances like aligners, which are made from polymer-based materials. When subjected to continuous mechanical forces, such as chewing, tooth movement, or cleaning, these materials can wear down, leading to the release of microplastic particles.

Clear aligners, made from polymers such as PET, PU, and PC, are susceptible to mechanical stress, leading to the breakdown of these materials into MPs. The presence of secondary MPs in clear aligners is particularly concerning due to their widespread use in both adult and pediatric orthodontic treatment.

The study investigated the release of MPs from clear orthodontic aligners (Invisalign) due to mechanical friction under *in vitro* conditions, using artificial saliva to simulate oral environments. This study provides important insights into the potential environmental and health impacts of clear aligners, focusing on the quantity, size, and composition of MPs released under mechanical friction, which mimics physiological tooth movements.

Raman Microspectroscopy (RMS) and Scanning Electron Microscopy (SEM) were chosen as the primary methods to analyze the released MPs due to their unique advantages in polymer identification and particle characterization. Raman spectroscopy provides a highly sensitive and precise way to detect the chemical composition of the MPs. One of the key advantages of Raman over other techniques such as IR spectroscopy is its ability to analyze small particles with high spatial resolution, making it ideal for distinguishing the chemical structure of MPs, which often absorb contaminants *in vivo*.[15] Furthermore, SEM complements this by providing high-resolution images to assess the morphology and size of the particles.

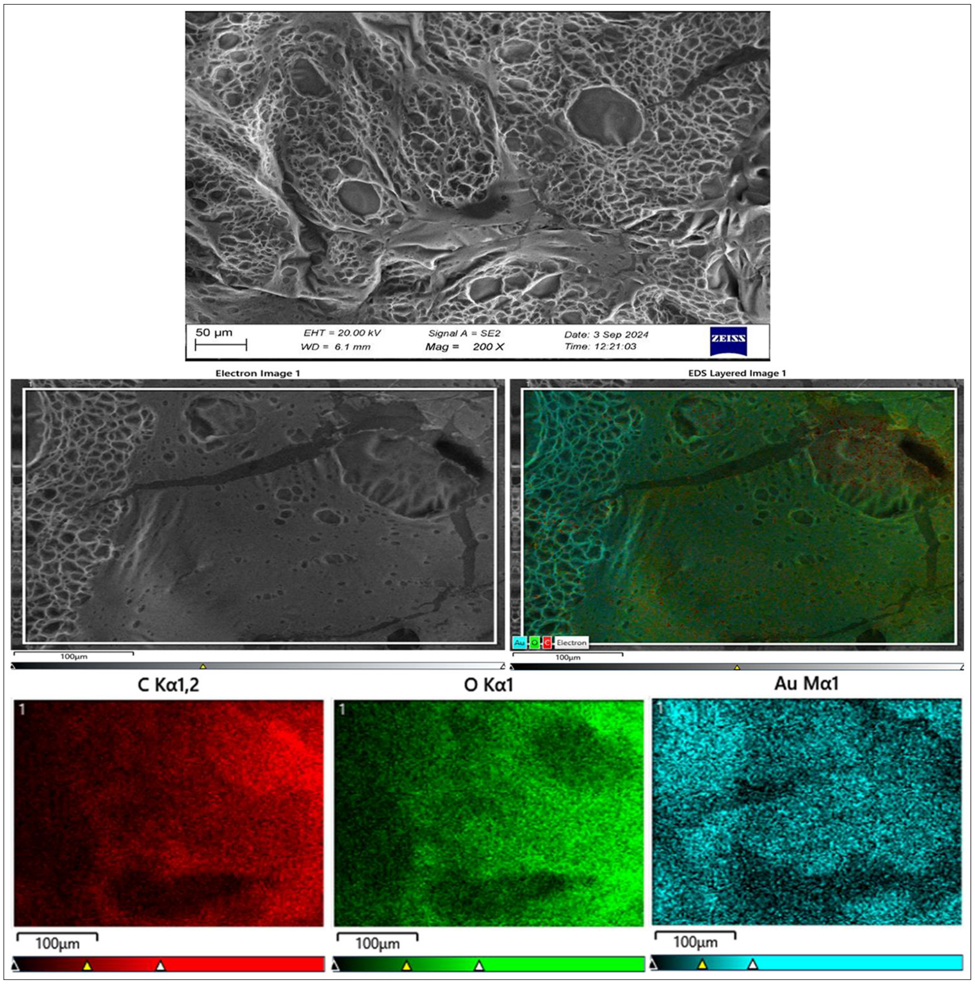
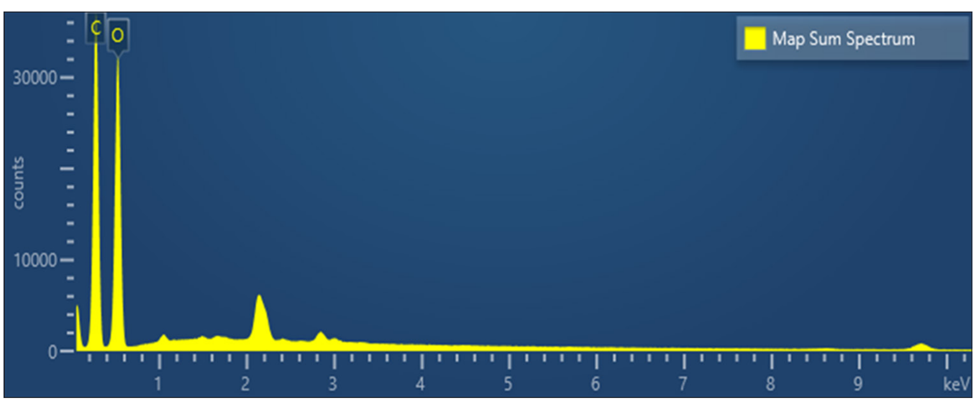


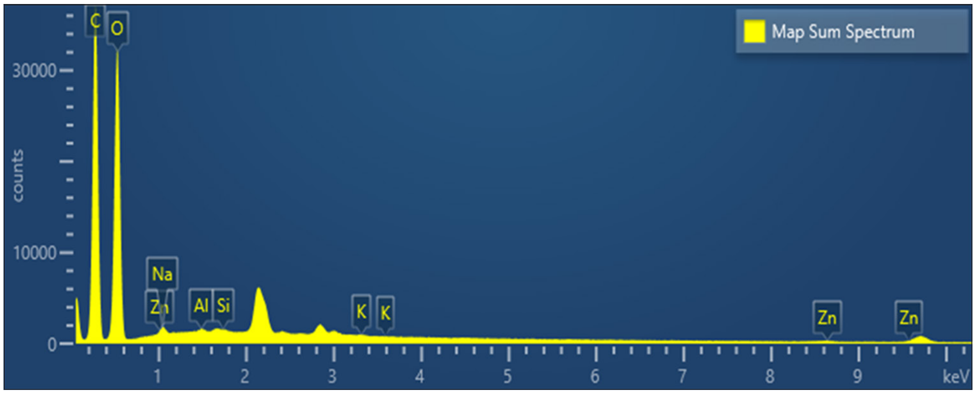
Figure 8: (a) Microplastic fiber detected in Scanning electron Microspectroscopy with various elements, (b) Carbon (C), (c) oxygen (O_2), silicon (Si), (d) sodium (Na), aluminum (Al), and potassium (K).



Graph 1: Representation of elements such as carbon (C) and oxygen (O) in scanning electron microscopy (SEM).

Together, these techniques give a comprehensive view of the MPs, confirming both their polymeric nature and their physical characteristics, which are critical for understanding their potential toxicity and environmental persistence.

The results from this study confirmed the detachment of MPs from clear aligners under various durations of mechanical friction, which consists of the control group, which was kept in artificial saliva without mechanical friction, showed no significant release of MPs. This suggests that static exposure



Graph 2: Representation of elements such as sodium (Na), aluminium (Al), silicone (Si), potassium (k), and zinc (Zn).

to artificial saliva is not sufficient to cause microplastic detachment. In contrast, the aligners subjected to mechanical friction for 3, 5, 7 h, and 7 days showed an increasing release of microplastic particles. Specifically, after 3 h of mechanical friction, a small but detectable number of MPs were found, while 5 and 7 h showed a greater release, with the highest number detected after 7 days of friction.

The increasing number of MPs released over time confirms that mechanical forces similar to natural tooth movement are a significant factor in the detachment of MPs from clear aligners. Moreover, SEM analysis demonstrated the presence of microplastic fibers, with elemental analysis revealing the presence of C, O_2, Si, Na, Al, K, and Zn, all of which are commonly found in polymeric dental materials and environmental contaminants.

RMS confirmed the composition of these MPs, matching them to the polymeric matrix of the clear aligners, primarily made from PU. This highlights a potential health risk since prolonged use of these aligners could result in continuous exposure to MPs, with unknown long-term health effects.

In 2021, according to Katras *et al*., there is a possibility that the three types of orthodontic aligners under investigation will be released for bisphenol-A (BPA). The amount of BPA released is significantly less than the safety thresholds set for adult patients. There was no discernible variation in the amount of BPA released across the three sets of aligners in each of the three media. Most BPA releases happened within the first 24 hours of the aligners being embedded. The concern is compounded by the fact that aligners are typically worn for extended periods – up to 22 hours a day for several years, as treatment can last 3–5 years. This extended wear leads to chronic exposure to MPs, which may pose significant health risks. Prolonged exposure to these MPs could lead to chronic inflammation, immune system disruptions, and even an increased risk of cancer, as the body struggles to expel these particles. In addition, the presence of chemicals like bisphenol A (BPA), often found in plastic-based products, raises concerns about potential endocrine disruption. BPA and similar chemicals are known to interfere with the endocrine system, potentially causing hormonal imbalances and long-term reproductive and developmental health issues. In conclusion, wearing aligners for extended periods (22 h daily over 3–5 years) can expose individuals to significant amounts of MPs, which may act as endocrine disruptors, contributing to hormonal dysregulation and other long-term health risks. This underscores the need for further research into the materials used in aligners and the long-term effects of their chronic exposure.[18]

The study by Quinzi *et al*. systematically simulated the mechanical friction that aligners experience in the oral cavity by stirring the aligners immersed in the artificial saliva. The results demonstrated that mechanical friction over time leads to the release of MPs, which could potentially be ingested by users.[4] Using liquid chromatography/mass spectrometry techniques (LC-ESI-MS/MS for urethane Di methacrylate [UDMA] and LC-APCI-MS/MS for BPA), A Willi *et al*. quantitatively evaluated the degree of conversion and the water-leaching targeted component from 3D-printed aligners. Finished despite being effectively polymerized and free of BPA, the wide range in the quantity of UDMA monomer that leached from the samples under examination raises questions regarding possible health risks during repeated intraoral exposure, which is recommended for this class of materials.[19]

The study that investigated the cytotoxic and estrogenic effects of Invisalign appliances by Eliades *et al*. The researchers analyzed the release of potentially harmful substances from these aligners and their impact on human health. Findings indicated that certain chemical components in Invisalign aligners could leach out, raising concerns about long-term exposure. The study emphasized the need for further research into the biocompatibility of such orthodontic devices.[20]

The *in vitro* cytotoxicity of the direct-printed aligner employing photopolymer resins and the Smart Track Invisalign tray was studied by Ahamed *et al*. on 3T3 mouse fibroblast cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay at different time intervals. The most biocompatible material found for Invisalign was PU, which was used in the Smart Track version, and polymethyl methacrylate, which was used in the directly printed aligners. All materials showed a greater degree of cytotoxicity on the first day, which progressively decreased over the following days. While there is just a small amount of cytotoxicity, the data show that there was increased material leaking during the first use.[21]

When Kolesti D evaluated the surface roughness of Invisalign[R] appliances in the retrieved and "as-received" control states, as well as between the control and retrieved 3D-printed groups, they found differences in surface roughness between the retrieved and retrieved InvisalignR appliances.[22] This analysis is relevant to our study on microplastic release because surface roughness directly affects the rate and extent of material degradation. The rougher the surface of an aligner, the more susceptible it is to mechanical wear during chewing, tooth movement, and cleaning. Increased surface roughness may lead to a higher rate of microplastic particle detachment due to friction. Thus, differences in surface texture between retrieved and control aligners, as well as between traditional and 3D-printed aligners, can influence the quantity and characteristics of MPs released during long-term use. This ties into our findings, as prolonged mechanical friction in the oral environment leads to microplastic release, and aligners with rougher surfaces could exacerbate endocrine issues.[2]

Limitations

Despite the significance of these findings, several limitations should be acknowledged. First, this is an *in vitro* study, and while the tribiometer simulates physiological conditions, actual oral environments are more complex. Saliva, food particles, and bacterial activity may all contribute to microplastic degradation and release, factors that were not fully accounted for in this study. In addition, the study focused on only one brand of clear aligners (Invisalign), and the generalizability of these findings to other brands or materials is unclear.

Future recommendations

Future studies could address these limitations by conducting long-term *in vivo* studies to evaluate the release of MPs in real-life conditions. It would also be valuable to investigate whether certain materials or manufacturing techniques reduce the risk of microplastic release. Another key area for future research is the development of more biocompatible materials that are resistant to mechanical wear or biodegradable to mitigate environmental and health impacts.

CONCLUSION

This study highlights the release of MPs from clear orthodontic aligners due to mechanical friction, simulating conditions similar to those in the oral cavity. The results show that significant microplastic release occurs after prolonged mechanical friction, raising concerns about the potential health and environmental impacts. The composition of these MPs, primarily PU, and their detection through Raman Microscopy and SEM confirm that the polymeric matrices of aligners degrade under stress, releasing MPs into the oral environment. Given that aligners are worn for extended periods, the risk of chronic exposure to MPs is significant and could lead to long-term health effects, including chronic inflammation and endocrine disruption. The study calls for further research into biocompatible materials to reduce microplastic release and *in vivo* studies to evaluate real-world exposure and impacts on human health. The study concluded that prolonged mechanical friction, simulating physiological teeth movement, results in increased detachment of MPs from clear aligners, with both the number and size of particles increasing over time.

Ethical approval: The Institutional Review Board has waived the ethical approval for this study. Waiver number: 371

Declaration of patient consent: Patient consent was not required as there are no patients in this study.

Financial support and sponsorship: Nil.

Conflicts of interest: There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation: The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript, and no images were manipulated using AI.

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