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Can wear time, material composition, and manufacturing processes affect microplastic release from clear orthodontic aligners? A multidisciplinary *in vitro* study

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Objective: Plastics are widely used, including in medical applications. In dentistry, clear aligners (CAs) offer an alternative to fixed appliances, but their potential microplastics (MP) release raises health concerns. This study aims to investigate the detachment of MPs from CAs by analyzing the correlations between wearing time, manufacturing methods, and material composition. **Methods:** The following CAs were tested: Alleo (AL), F22 Aligner (F22), FlexiLigner (FL), Graphy (GP), Invisalign (INV), KeySplint (KS), Lineo (LIN), LuxCreo (LC), Spark (SP), and SureSmile (SS). One pair of CAs per group was immersed in artificial saliva, and the solution was stirred for 5 hours per day for 7 (T1) or 14 (T2) days to simulate mechanical friction. Saliva samples were subsequently vacuum-filtered through 1.6- μ m pore-size membranes. Attenuated total reflectance-Fourier transform infrared spectroscopy was used to characterize the CA materials, and Raman microspectroscopy and scanning electron microscopy were used to assess the chemical composition, number, size, and shape of the MPs. The experiment was conducted in triplicate. **Results:** The release of MPs increased significantly from T1 to T2 ($P < 0.05$), especially those with a diameter $< 5 \mu$ m. The mean size of the MPs did not significantly differ at either time point. MP release was associated with material composition at T1 and T2 ($P < 0.0001$). No significant correlation was observed with the manufacturing process, although GP, KS, and LC, which are three-dimensionally printed (3D-printed) CAs, released more uniform and spherical MPs. **Conclusions:** Replacing CAs after 7 days may help limit MP release, particularly smaller fragments; however, the clinical significance of this finding remains unclear and requires further *in vivo* investigation.

Keywords: Aligner, Scanning electron microscopy, Public health, Biomaterial science

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INTRODUCTION

Plastic use has become pervasive across industrial, medical, commercial, and personal care sectors,¹ making plastic contamination a global concern due to its adverse effects on human health and environmental safety.²

Microplastics (MPs), defined as plastic debris 1 μm –5 mm in length, occur in granular, fibrous, and film-like forms³ and are classified as primary (intentionally produced at micrometric size) or secondary (derived from fragmentation of larger plastics).⁴ Humans may ingest nearly 55,000 microparticles annually through seafood alone.⁵ MP size, shape, and composition influence cellular uptake and intracellular fate.⁶ MPs can enter the bloodstream^{7,8} and reach tissues including lungs, arterial plaques, placenta, breast milk, and sperm, with variable distribution.^{2,8-12} They may also cross the blood–brain barrier, accumulate in neural tissue, and potentially contribute to neuroinflammation, neurodegenerative diseases, and cognitive impairment.¹³⁻¹⁷

Plastics are widely used in dentistry, with applications ranging from oral care products to restorative materials and orthodontic appliances.¹⁸ Clear aligners (CAs) have played a particularly important role in revolutionizing orthodontics by offering a removable, discreet, and patient-friendly alternative to conventional appliances.¹⁹ Patients are typically instructed to wear their aligners for approximately 22 hr/day, replacing it every 7–14 days.²⁰ Historically, CAs have been fabricated using a conventional method based on vacuum thermoforming, whereby thermoplastic materials are molded onto physical models.²¹ The materials used in aligners production include thermoplastic polymers, polymer blends, three-dimensionally printed (3D-printed) materials, and bioactive materials.²⁰ The polymers used predominantly include polyvinyl chloride, polyethylene terephthalate (PET), polyethylene terephthalate glycol, polypropylene, polycarbonate, and polyurethane (PU) owing to their excellent mechanical and optical properties.²²

Despite the clinical advantages of aligners, there has been growing concern regarding the impact of CA-derived MPs. Our group first demonstrated in an *in vitro* study that MPs detach from CAs through mechanical friction,²³ and we characterized their size, shape, and number. These findings were indicative of the potential health risks associated with CA use.²⁴⁻²⁶ However, given the limited literature that is currently available, a deeper understanding of CA behavior is required, particularly regarding the impact of plastic composition, manufacturing methods, and wearing times. Clarifying how these factors affect MP release and their possible correlations could influence clinical protocols, inform public health strategies, and guide the development of improved ma-

terials.

Therefore, the aim of the present study was to investigate the release of MPs by CAs *in vitro* by analyzing the correlations between wearing time (7 or 14 days), manufacturing methods (thermoforming or direct 3D-printed), and material composition. The chemical composition of the CAs was analyzed using attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy, and the number and complete morphological and chemical characterization of the released MPs were determined using Raman microspectroscopy (RMS) and scanning electron microscopy (SEM).

MATERIALS AND METHODS

Sample collection

Orthodontic CAs were obtained from the following manufacturers (Table 1): Alleo (AL, Leone S.p.a, Firenze, Italy), F22 Aligner (F22, Sweden and Martina Company, Padua, Italy), FlexiLigner (FL, FlexiLigner S.r.l, Roma, Italy), Graphy (GP, Graphy Inc., Seoul, Korea), Invisalign (Align Technology Inc., San Jose, CA, USA), KeySplint (KS, Keystone Europe LLC, Batavenweg, Netherlands), Lineo (LIN, Micerium S.p.a, Genova, Italy), LuxCreo (LC, LuxCreo Inc., Chicago, IL, USA), Spark (SP, Ormco Corporation, Brea, CA, USA), and SureSmile (SS, Dentsply Sirona, York, PA, USA). Moreover, a control group (CTRL) was included, in which the CAs were not fractioned to exclude the potential for MP detachment caused by other factors such as temperature and suspension in saliva rather than mechanical friction.

For each manufacturer, three pairs of CAs derived from the same stereolithography file were subjected to a mechanical friction protocol for each time point: 7 days (T1) and 14 days (T2). Each pair of CAs was immersed in a glass beaker containing 50 mL of artificial saliva (Biotène Oral Balance, GSK, Brentford, United Kingdom). Throughout the experiment, the beakers were covered with aluminum foil to avoid environmental contamination and placed onto a magnetic hot plate (SuperNuova+™ Stirrer series, Thermo Scientific™, Loughborough, UK) to maintain a constant temperature of 37°C. A Teflon-coated, cylindrical magnetic stirring bar (6 mm × 25 mm) was used to generate a rotating magnetic field for 5 hr/day to simulate the friction that occurs between teeth during swallowing. Given a spontaneous swallowing rate of 0.98/min²⁷ and an aligners wear time of 20–22 hr/day,²² the daily number of swallows was determined to be approximately 1,235. Thus, the stirring bar was calibrated at 250 rotations/hr, yielding 1,250 rotations in the daily 5-hour simulation.

At the predetermined time points (T1 and T2), the artificial saliva samples were filtered through 1.6- μm pore-size membranes (Whatman GF/A, 47-mm diameter)

Table 1. Details of the orthodontic clear aligners analyzed in this study, including the name of the brand, commercial manufacturer, material composition, and manufacturing method

Brand	Commercial manufacturer	Material composition	Manufacturing method
Alleo (AL)	Leone S.p.a, Firenze, Italy	Polyethylene terephthalate glycol	TA
F22 Aligner (F22)	Sweden and Martina Company, Padua, Italy	F22 Polyurethane	TA
FlexiLigner (FL)	FlexiLigner S.r.l, Roma, Italy	Polyethylene terephthalate	TA
Graphy (GP)	Graphy Inc., Seoul, Korea	Graphy's resin, Tera Harz TC-85 Photopolymerizable polyurethane, based on acrylic esters, Methacrylate-based resins	DPA
Invisalign (INV)	Align Technology Inc., San Jose, CA, USA	SmartTrack™ Material; Multilayer aromatic polyurethane from methylene diphenyl diisocyanate and 1,6-hexanediol plus additives	TA
KeySplint (KS)	Keystone Europe LLC, Batavenweg, Netherlands	KeyPrint, photopolymer resin	DPA
Lineo (LIN)	Micerium S.p.a, Genova, Italy	Polyethylene terephthalate glycol-based	TA
LuxCreo (LC)	LuxCreo Inc., Chicago, IL, USA	DCA resin: Polyurethane methacrylate A, Polyurethane methacrylate B, Isobornyl methacrylate, 4-Acryloylmorpholine	DPA
Spark (SP)	Ormco Corporation, Brea, CA, USA	TruGEN™ and TruGEN XR, polyurethane-polyester	TA
SureSmile (SS)	Dentsply Sirona, York, PA, USA	Essix Plastics: Plus, C plus, Polypropylene/ethylene copolymer (> 95%), stabilizers (< 5%), Polyurethane resin	TA

TA, thermoformed aligner; DPA, direct three-dimensionally printed clear aligner; DCA, dual-cure acrylate.

using a vacuum pump connected to a filtration system. The membranes were subsequently air-dried at room temperature and placed in glass Petri dishes until further RMS and SEM analysis. For each manufacturer, three independent maxillary and mandibular pairs of aligners were tested at T1 and another three were evaluated at T2. Each pair was processed in a separate beaker. These were true material replicates, not technical repeats, thereby ensuring reproducibility without reusing the same specimens.

ATR-FTIR spectroscopy measurements

CAs were analyzed using ATR-FTIR spectroscopy to determine their polymer composition. Analyses were performed at the ARI (Advanced Research Instrumentation) Laboratory, using a platinum ATR accessory with a diamond crystal, coupled to a Bruker INVENIO-R interferometer equipped with a deuterated triglycine sulfate detector (Bruker Optics, Ettlingen, Germany). Each sample was pressed onto the crystal, and three spectra were collected from different areas at room temperature. The spectra were recorded from 4,000–700 cm^{-1} with 4 cm^{-1} resolution, generating an average of 256 scans. A back-

ground spectrum was acquired before each measurement. The following pre-processing steps were performed on the raw spectra using OPUS 7.5 software (Bruker Optics): correction for atmospheric CO_2 and H_2O levels, vector normalization, trimming to 1,850–600 cm^{-1} , and two-point baseline correction. Polymer identification was performed by comparing the spectra with those in the OPUS library; hit quality index (HQI) values > 80 were considered satisfactory.

RMS measurements

The RMS analyses were performed at the ARI Laboratory using an XploRA Nano Raman Microspectrometer (Horiba Scientific, Sunnyvale, CA, USA). Before use, all filter membranes, including procedural blanks, were inspected under visible light at a 10× objective (Olympus MPLAN10×/0.25, Olympus Corporation, Tokyo, Japan). MPs were morphologically characterized at a 100× objective (Olympus MPLAN100×/0.90) and subsequently analyzed using RMS (200–1,800 cm^{-1} ; 532 or 785 nm laser; 600 lines/mm grating). Spectra were collected using a Peltier-cooled 16-bit charge coupled device detector, and the instrument was calibrated to the 520.7 cm^{-1}

silicon band. Raw Raman spectra were processed using polynomial baseline correction and vector normalization (LabSpec 6, Horiba Scientific). Polymer identification was performed by comparing the spectra with reference libraries of standard polymers (KnowItAll, John Wiley & Sons, Inc., Hoboken, NJ, USA);²³ HQI values > 80 were considered satisfactory.

SEM characterization

The same filter membranes that were analyzed using RMS were subsequently subjected to SEM analysis at the Centre for Electron Microscopy (CISMIN, Centro di Ricerca e Servizio di Microscopia delle Nanostrutture). A portion of the filter, approximately 10 × 10 mm, was cut in the central part of each filter membrane, mounted on aluminum stubs, sputter-coated with gold, and observed under a TESCAN VEGA 3 LMU SEM (TESCAN ORSAY HOLDING, Brno, Czech Republic), operated at 15 kV and at variable working distance with a secondary electron detector. Following the methodology reported in our prior study, SEM imaging was conducted at multiple magnifications (350×–5.80k×) to analyze the number, shape, and size of the MPs.²³

Quality assurance and control

To prevent environmental contamination, a plastic-free protocol was adopted. Cotton lab coats and latex gloves were worn, and all friction and filtration procedures were conducted in a dedicated room. Plastic tools were replaced with detergent-cleaned glassware rinsed with 70% ethanol and 1.6 µm–filtered deionized water. Surfaces were disinfected with 70% ethanol before and during procedures. Environmental and procedural blanks were included to detect MP contamination. For environmental blanks, a filter membrane soaked in filtered deionized water was placed daily in an uncovered Petri dish in the dedicated room and later examined under a stereomicroscope.

Statistical analysis

For each group, the number and size of the MPs are expressed as the means of three replicates at each time point. Normally distributed size data are reported as the mean ± standard deviation. Significant intergroup differences were assessed using one-way and two-way analyses of variance (ANOVAs), followed by Tukey's post hoc test. Two-way ANOVA was used for intergroup comparisons of the number and size of the MPs at T1 and T2 and to infer population means. The size distribution (%) of the MPs across three ranges (< 5 µm, 5–20 µm, and > 20 µm) was also evaluated. Statistical significance was set at $P < 0.05$, and the analyses were conducted using Prism 6 software (GraphPad Software, San Diego, CA, USA).

Pearson correlation analysis was performed to examine the relationships between the mean number of detached MPs and the two time points (T1 and T2). Correlation coefficients (r) ranged from –1 to 1, with values closer to ±1 indicating stronger linear relationships. Linear regression models were fitted for each time point, and R^2 values were calculated to assess the model fit, with values approaching 1 indicating better agreement. Scatter plots with regression lines were used to visualize the correlations at each time point.

RESULTS

No MP detachment was observed in the CTRL filters, confirming that their release was caused by friction rather than other factors.

ATR-FTIR spectroscopic analysis was first conducted to characterize the chemical composition of the tested CAs; representative IR spectra are shown in Figure 1A. Specifically, the GP, KS, and LC aligners were mainly composed of polymethyl methacrylate (PMMA), with bands observed at ~1,710 cm^{-1} (stretching vibration of the carbonyl ester group $\text{C}=\text{O}$), ~1,240 cm^{-1} (stretching vibration of $\text{C}-\text{CO}$ moieties), and ~1,149 cm^{-1} and ~1,103 cm^{-1} (stretching vibrations of $\text{C}-\text{O}$ bonds).²⁸ The F22 and INV aligners were composed of PU, with bands at ~1,699 cm^{-1} (stretching vibration of the carbonyl amide group $\text{C}=\text{O}$), ~1,520 cm^{-1} (stretching vibration of the $\text{C}-\text{N}$ bond and bending vibration of the $\text{N}-\text{H}$ bond), ~1,217 cm^{-1} (stretching vibration of the $\text{C}-\text{O}-\text{C}$ moiety), and ~1,065 cm^{-1} (stretching vibration of $\text{C}-\text{O}$ bonds).²⁹ The AL, FL, and LIN aligners exhibited the same spectral profiles compatible with PET, with bands detected at ~1,714 cm^{-1} (stretching vibration of the carbonyl ester group $\text{C}=\text{O}$), ~1,408 cm^{-1} (benzyl ring vibration), ~1,244 cm^{-1} (stretching vibration of $\text{C}-\text{CO}$ bonds), ~1,092 cm^{-1} (stretching vibration of $\text{C}-\text{O}$ bonds), and ~725 cm^{-1} (out-of-plane bending vibration of the benzene ring).³⁰ Finally, the SP and SS aligners were a mix of polyesters and PU.

The CAs were subsequently subjected to a mechanical friction protocol for T1 or T2, and the detached MPs were investigated using RMS and SEM analyses to characterize their chemical composition and morphology (i.e., their number, size, and shape). Figure 1B shows the Raman spectra and corresponding micrographs of representative MPs detached from the tested CAs. All ATR-FTIR spectroscopy and RMS identifications exhibited HQI values ≥ 80%, confirming reliable polymer assignment and correspondence between the aligners and detached MPs (Supplementary Table 1). SEM analysis was performed to obtain information on the number, size, and morphology of the detached MPs. Figure 2 shows representative SEM micrographs of the MPs collected

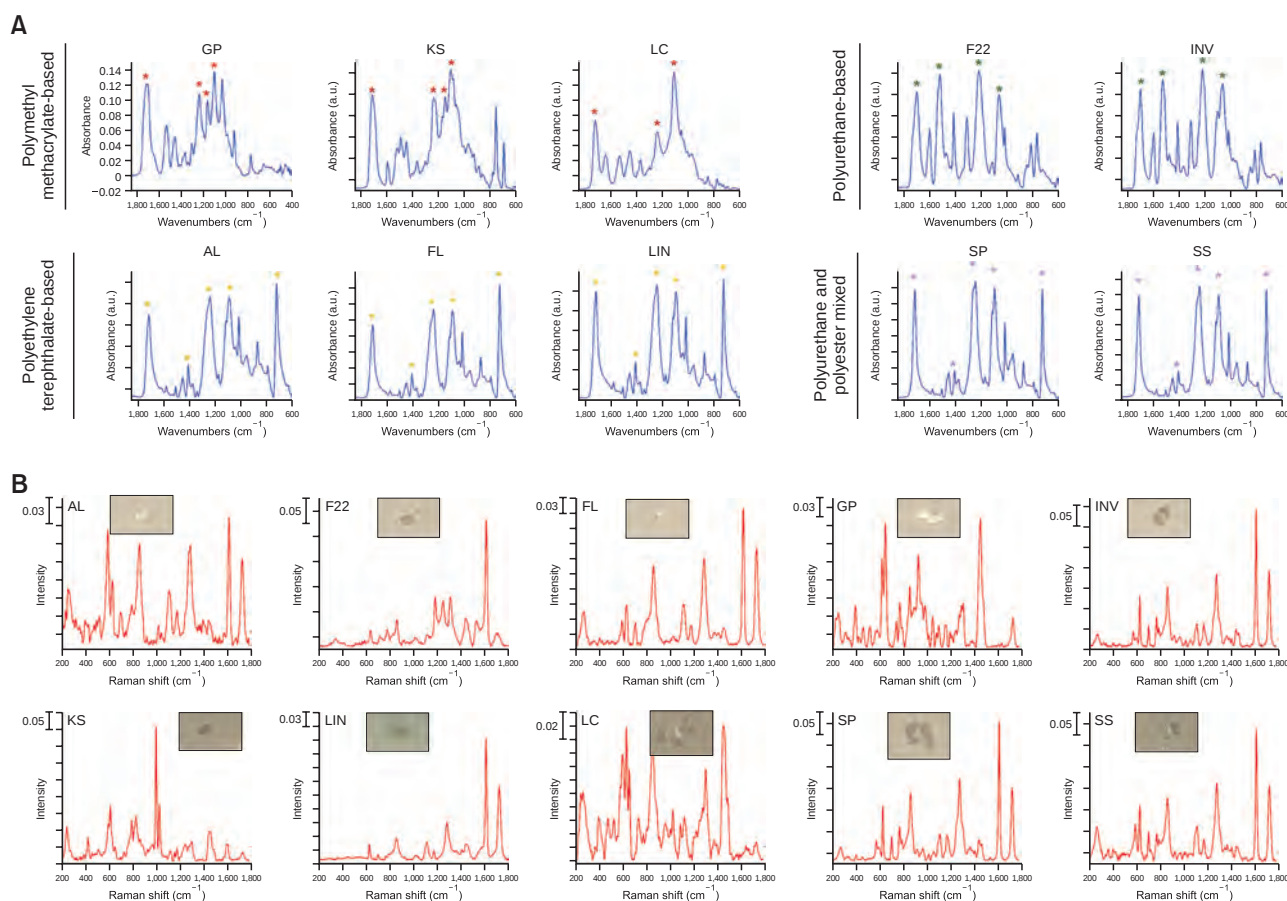


Figure 1. Spectroscopic analyses. **A**, Representative IR spectra collected on the tested clear aligners (CAs). Asterisks indicate the most significant bands of the following polymer matrices: polymethyl methacrylate (PMMA) (red), polyurethane (PU) (green), polyethylene terephthalate (PET) (orange), and PU/polyesters (pink). **B**, Raman spectra collected on microplastics detached from the tested CAs, with corresponding micrographs. Images were collected at a $\times 100$ objective (Olympus MPLAN100 $\times$ /0.90).

AL, Alleo; F22, F22 Aligner; FL, FlexLigner; GP, Graphy; INV, Invisalign; KS, KeySplint; LIN, Lineo; LC, LuxCreo; SP, Spark; SS, SureSmile.

from each group at T1 and T2, and Table 2 shows the mean number of MPs collected from each CA at T1 and T2, as well as the mean size and the size of the smallest and largest MPs. Notably, no MPs were found in either the environmental or procedural blanks.

At T1, the AL, FL, and LIN CAs released the highest number of MPs (> 16 MPs), whereas the GP aligners released the fewest (~ 7 MPs) (Figure 3A). Similar results were observed at T2, with the AL, FL, KS, and LIN CAs releasing the most MPs (> 36 MPs) and the GP aligners releasing the fewest (< 18 MPs) (Figure 3B). A statistically significant increase in the number of detached MPs was always found moving from T1 to T2 for every CA ($P < 0.0001$) (Figure 3C); however, the greatest increase (> 20 MPs) between the two time points was observed for the AL, FL, LIN, KS, and LC CAs, whereas the lowest increase (< 15 MPs) was observed for the F22, INV, GP,

SS, and SP CAs.

No significant differences in the mean MP size were observed among the CAs at either time point (T1 or T2) ($P > 0.05$) (Figure 3D and 3E); SP aligners exhibited the largest MPs at T1 ($38.95 \pm 10.47 \mu\text{m}$). The comparison of the MP size within each CA between T1 and T2 revealed the same trend, with no significant differences ($P > 0.05$), except for the SP aligners ($P < 0.05$) (Figure 3F). Interestingly, as the exposure time increased from T1 to T2, the mean size of MPs decreased in all groups, with the exception of the LIN aligner, although the change was not statistically significant ($P > 0.05$). Only the SP aligners demonstrated statistically significant differences between the mean MP size at T1 ($38.95 \pm 10.47 \mu\text{m}$) and T2 ($14.36 \pm 7.68 \mu\text{m}$) ($P < 0.05$).

The Pearson correlation analysis revealed a strong and significant positive relationship between the number of

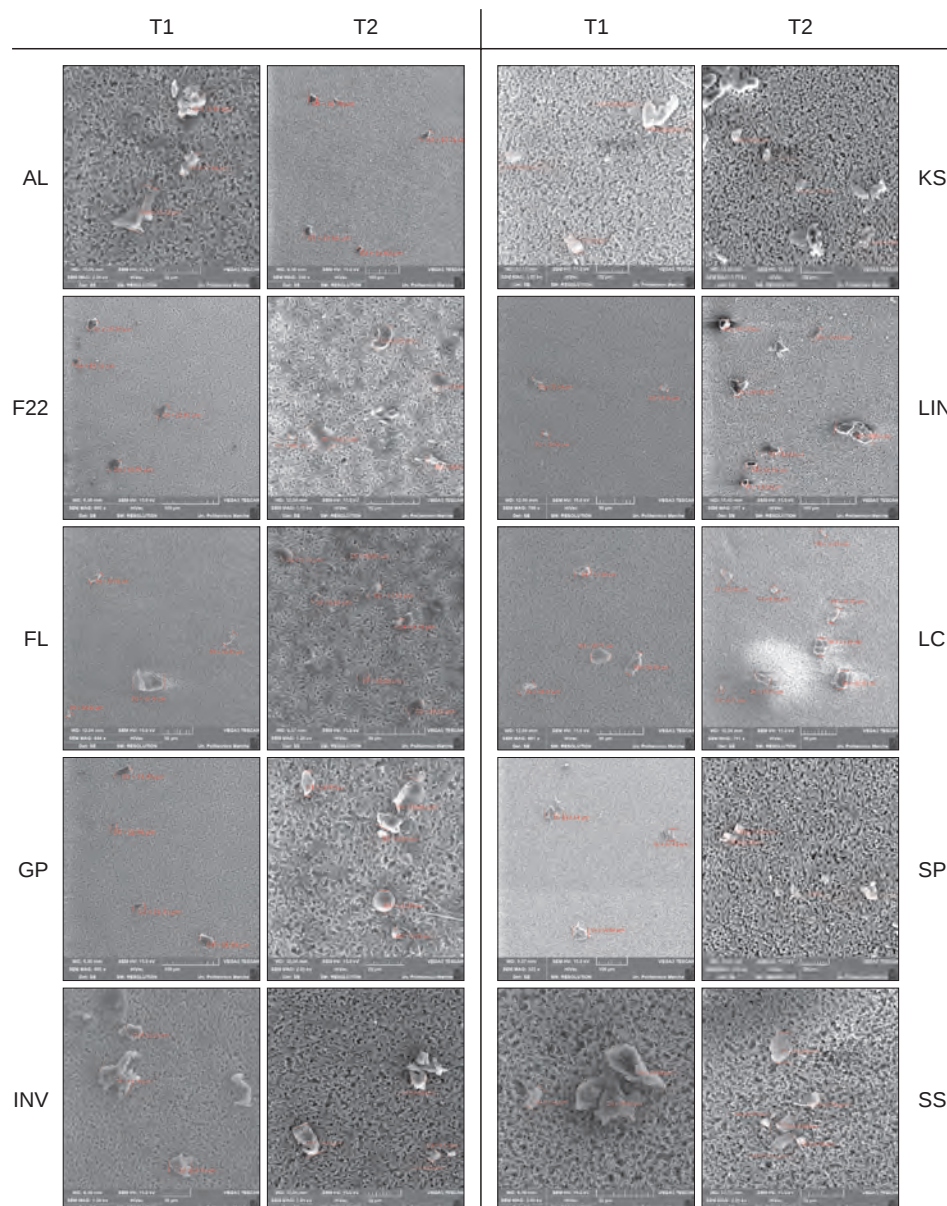


Figure 2. Representative scanning electron micrographs. Images collected at different magnifications (from 350x to 2.25kx) on selected microplastics detached at T1 (7-day protocol) and T2 (14-day protocol) from the following aligners: AL (Alleo); F22 (F22 Aligner); FL (FlexiLigner); GP (Graphy); INV (Invisalign); KS (KeySplint); LIN (Lineo); LC (LuxCreo); SP (Spark); SS (SureSmile). Size measurements are shown in red.

MPs released at T1 and T2 ($r = 0.9308$, $R^2 = 0.8663$, $P < 0.0001$; **Figure 3G**). Higher MP counts were observed for the AL, FL, and LIN aligners (all of which were predominantly PET-based), whereas the GP and INV aligners (which were PMMA- and PU-based) exhibited the lowest counts. Intermediate levels were observed for the KS and LC (PMMA-based), SP and SS (polyester/PU mixes), and F22 (PU-based) aligners. No correlation was observed between MP size and the two time points (data not shown).

Figure 4 shows the trends of the smallest (**Figure 4A**) and largest (**Figure 4B**) MPs at T1 and T2 for the different CAs. With regard to smaller MPs, no significant differences between time points were observed for any CA ($P > 0.05$), only in SP, the smallest MPs measured at T1 and T2 were $22.09 \mu\text{m}$ and $4.04 \mu\text{m}$, respectively. This finding suggests that the maximum fragmentation with regard to the minimum MP size occurred at T1 for all CAs, with the exception of the SP aligner. Conversely, for larger MPs, even though a less homogeneous distri-

Table 2. Mean number and size of microplastics detached from clear aligners at T1 (7 days) and T2 (14 days)

Brand	Mean number*		Mean size* (μm)		Smallest (μm)		Largest (μm)	
	T1	T2	T1	T2	T1	T2	T1	T2
AL	16.67 ± 1.41	36.33 ± 2.08	18.61 ± 9.26	15.55 ± 8.33	4.22	4.07	39.56	37.26
F22	9.67 ± 2.52	24.67 ± 3.51	18.15 ± 7.88	14.87 ± 8.00	4.70	4.03	33.69	39.08
FL	16.00 ± 2.00	37.67 ± 2.52	17.57 ± 9.79	15.41 ± 7.74	4.22	3.90	50.79	38.85
GP	5.67 ± 1.15	17.67 ± 2.08	19.89 ± 6.46	14.84 ± 7.71	5.23	3.44	31.43	31.50
INV	6.67 ± 2.08	20.67 ± 2.52	16.23 ± 9.06	12.92 ± 6.68	4.13	3.65	38.62	29.44
KS	11.33 ± 0.58	36.00 ± 3.46	24.59 ± 9.95	15.91 ± 7.99	7.78	3.55	46.46	33.95
LIN	16.67 ± 2.31	41.00 ± 1.73	15.63 ± 7.38	17.47 ± 10.27	4.12	3.82	33.58	72.02
LC	11.67 ± 1.15	33.00 ± 1.73	20.14 ± 9.60	17.38 ± 7.74	5.09	4.29	44.79	35.73
SP	9.33 ± 2.52	23.33 ± 3.06	38.95 ± 10.47	14.36 ± 7.68	22.09	4.04	62.14	29.57
SS	9.00 ± 1.00	21.33 ± 2.52	15.81 ± 7.07	13.95 ± 7.13	5.62	4.22	31.48	29.62

*Mean number" and "Mean size" are presented as mean ± standard deviation.

AL, Alleo; F22, F22 Aligner; FL, FlexiLigner; GP, Graphy; INV, Invisalign; KS, KeySplint; LIN, Lineo; LC, LuxCreo; SP, Spark; SS, SureSmile.

*Mean values were the result of three replicates.

bution was detected, all groups demonstrated a slight decrease between T1 and T2, with the exception of the F22, LIN, and GP CAs ($P < 0.05$).

Figure 4C shows MP size distribution for each CA in three intervals ($< 5 \mu\text{m}$, $5\text{--}20 \mu\text{m}$, $> 20 \mu\text{m}$). At T1, the smallest MPs were found in AL ($4.48 \mu\text{m}$), F22 ($4.70 \mu\text{m}$), and INV ($4.35 \mu\text{m}$); KS, GP, LC, SS, and SP showed none $< 5 \mu\text{m}$. At T2, all groups exhibited MPs $< 5 \mu\text{m}$, with GP the smallest ($3.44 \mu\text{m}$).

At $2,000\text{--}5,000\times$, MPs mainly displayed fragmented morphology, as reported by Campanale et al. (Figure 5).³¹ INV, F22, SP, and SS formed microsphere aggregates; FL and LIN produced foil-like particles; GP generated pellet-like MPs; and KS and AL showed foam-like structures. Although mean MP size did not differ significantly among groups, all CAs showed increased MPs $< 5 \mu\text{m}$ from T1 to T2.

DISCUSSION

CAs, composed of plastic polymers, have transformed orthodontic treatment. However, their degradation and MP release during use warrant investigation. In 2023, our group first reported MP detachment from CAs after 7 days of mechanical friction,²³ and a subsequent review confirmed increasing evidence and the need to mitigate related health and environmental risks.²⁶ Despite these findings, manufacturers prioritize environmental sustainability over intraoral health implications.³² Although daily MP exposure from food, water, and air exceeds that from intraoral devices,³³ evidence in dentistry remains limited.

This study expands on previous findings by examin-

ing MP release from CAs in relation to their wear time (T1 vs. T2), polymer composition, and manufacturing method using ATR-FTIR spectroscopy, RMS, and SEM. The combined use of ATR-FTIR and RMS strengthened the data interpretation, as ATR-FTIR spectroscopy facilitated the classification of each aligner by polymer type, whereas the RMS analysis identified the chemical signatures of individual MPs. For all aligners, the RMS spectra of the detached MPs matched the polymer classes identified via ATR-FTIR spectroscopy, confirming that the released particles originated from the aligners themselves.

The results revealed a significant increase in the number of MPs released from T1 to T2, along with a reduction in size over the same time points, with particles $< 5 \mu\text{m}$ becoming predominant at T2. It is important to note that the measurements and imaging conducted at the T1 and T2 time points were based on independent experimental lines using the new aligners and filters. Although this design was employed to ensure reproducibility, it did not allow for sequential monitoring of the same sample; consequently, it cannot be determined from the present data whether the smaller MPs observed at T2 were derived from the secondary fragmentation of particles released at T1 or from the *de novo* detachment of smaller particles. This could represent a limitation of this study; thus, future studies using time-series sampling with real-time particle tracking should address this issue.

Most of the detected MPs were $5\text{--}20 \mu\text{m}$, a size that is generally more likely to be excreted via the gastrointestinal tract. Only a smaller fraction of MPs was $< 5 \mu\text{m}$, the size range more prone to epithelial translocation. MP toxicity depends on size, shape, and compo-

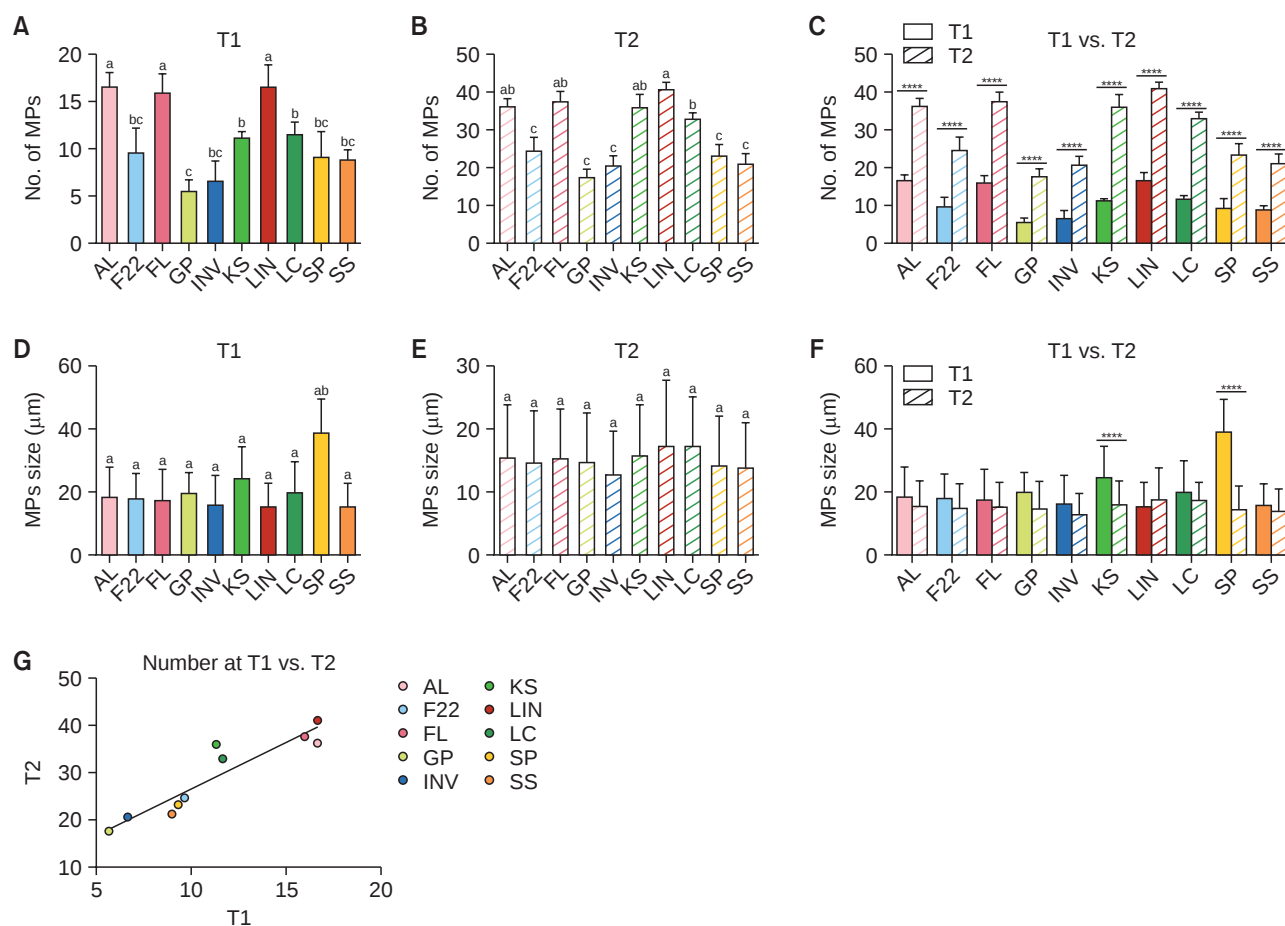


Figure 3. Assessment of the number and size of microplastics (MPs). Comparison of the mean number of MPs detected at (A) 7 days (T1) and (B) 14 days (T2). C, Comparison of the mean number of MPs for each CA between T1 and T2. D, Mean MP sizes observed at T1 and (E) T2 for each aligner. F, Comparison of the mean size of MPs detached from each aligner between T1 and T2. Data are presented as the mean $\pm$ standard deviation. Statistical significance was evaluated via one-way and two-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test (Prism 6 software; GraphPad Software, San Diego, CA, USA). Statistical significance was set at $P < 0.05$. Lower-case letters above the histograms indicate statistically significant differences among all CAs, whereas asterisks (****) indicate statistically significant differences between T1 and T2 for each CA ($P < 0.05$). G, Pearson correlation between the mean number of MPs and the two time points (T1 and T2).

AL, Alleo; F22, F22 Aligner; FL, FlexiLigner; GP, Graphy; INV, Invisalign; KS, KeySplint; LIN, Lineo; LC, LuxCreo; SP, Spark; SS, SureSmile.

sition,³⁴ as larger MPs are typically excreted, whereas particles $< 5 \mu\text{m}$ may cross epithelial barriers.³⁵ Although size did not correlate with the material composition or manufacturing method, all aligners exhibited the release of smaller particles over time, with more $< 5 \mu\text{m}$ fragments detected at T2 than at T1. This suggests that the 7-day wear protocol may be safer than the 14-day one in terms of MP exposure and is consistent with recent reviews that reported comparable orthodontic efficacy across 7-, 10-, and 14-day protocols, reinforcing the idea that changing aligners every 7 days could represent an optimal schedule.^{36,37} Although previous studies have

focused solely on orthodontic efficiency, these results collectively suggest that shorter wear protocols may offer both efficiency and potential safety advantages, although further research is needed.

CAs were classified as PMMA-based (GP, KS, LC), PU-based (INV, F22), PET-based (AL, FL, LIN), and mixed PU/polyester (SP, SS). MP release correlated with composition: PET-based aligners showed the highest detachment at T1 and T2, whereas SP and SS exhibited similar levels, likely due to shared polymers.

Manufacturing may influence material aging.²⁰ All CAs were thermoformed except GP, KS, and LC, which were

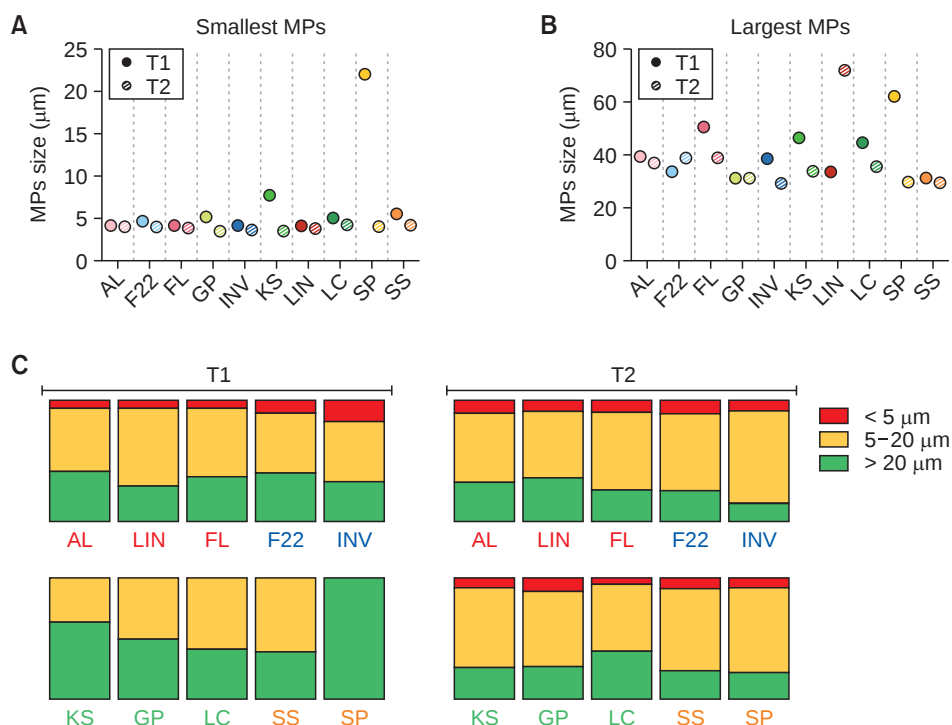


Figure 4. Microplastics (MPs) size. Trend of the smallest (A) and largest (B) MPs detected at T1 (after seven days) and T2 (after 14 days) for each clear aligner (CA). C, Distribution of the percentage (%) of the mean MP sizes, subdivided into three ranges (< 5 μm [red], 5–20 μm [yellow], and > 20 μm [green]) for each group and for each timing protocol (T1 and T2).

AL, Alleo; F22, F22 Aligner; FL, FlexiLigner; GP, Graphy; INV, Invisalign; KS, KeySplint; LIN, Lineo; LC, LuxCreo; SP, Spark; SS, SureSmile.

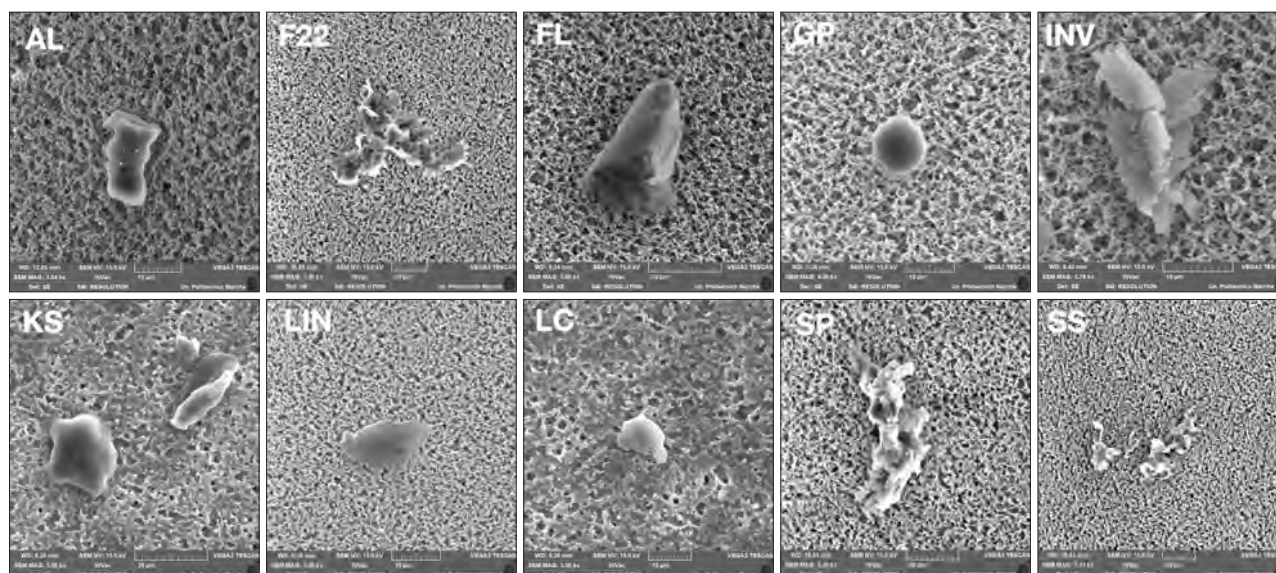


Figure 5. Representative SEM images, at magnifications ranging from 2,000 $\times$ to 5,800 $\times$, of microplastics detached from clear aligners: AL (Alleo); F22 (F22 Aligner); FL (FlexiLigner); GP (Graphy); INV (Invisalign); KS (KeySplint); LIN (Lineo); LC (LuxCreo); SP (Spark); SS (SureSmile). The majority of the MPs in all groups exhibited a fragment-like shape; however, the GP-derived MPs showed a pellet shape, the FL- and LIN-derived MPs appeared as a foil-like structure, and the KS- and AL-derived MPs exhibited a foam-like structure.

3D-printed. Confocal microscopy showed that seven days of intraoral use increased surface roughness and porosity in direct 3D-printed CAs compared with INV aligners.³⁸ However, no correlation emerged between MP number and manufacturing process; at T1, no MPs < 5 µm were detected in 3D-printed CAs. The GP aligner, PMMA-based and 3D-printed, showed the lowest MP detachment at both time points.

PMMA-based 3D-printed CAs released more spherical, regular MPs than F22, INV, SP, and SS, without significant differences. As MP toxicity correlates with surface roughness and inflammatory responses,³⁹ composition and manufacturing may influence MP morphology and potential toxicity after mechanical friction.

The use of 1.6-µm pore filters in this study followed established MP protocols, offering compatibility with Raman/SEM analyses and sufficient mechanical stability.^{12,23,40} However, particles between 1.0 and 1.6 µm in size may not be retained, representing a methodological limitation; therefore, the MP counts reported in this study likely represent conservative estimates. The main limitation of this study lies in attempting to reproduce, *in vitro*, the mechanical friction that occurs between dental arches during daily wear. Additional oral factors may further accelerate CA degradation, suggesting that the MP release detected in studies such as this is likely to be underestimated.⁴¹

Considering the limited experimental data on both this topic and MP metabolism in humans, the present findings should be interpreted with caution. Nonetheless, the results indicate that patients wearing CAs may be unknowingly exposed to MPs, highlighting the need for further research on innovative materials and wearing protocols to reduce the health risks associated with the release of MPs from intraoral devices.

CONCLUSIONS

This study assessed MP detachment from CAs according to wear time, composition, and manufacturing. Findings were: (1) MP release increased from T1 to T2, with more fragments < 5 µm; (2) GP and INV showed the lowest release at T1; (3) detachment correlated with composition; and (4) MP size did not differ significantly among groups.

Although release increased between days 7 and 14 of simulated wear, its clinical relevance remains unclear. Replacing aligners after 7 days may help limit MP release, particularly smaller fragments; however, this should be considered precautionary, and further *in vivo* and longitudinal studies are needed to clarify the health implications of prolonged CA use and to improve materials, manufacturing processes, and clinical protocols.

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AUTHOR CONTRIBUTIONS

Conceptualization: G Orilisi, GM, EG, G Orsini, VQ. Data curation: G Orilisi, FV, VT, SS, EG, G Orsini, VQ. Formal analysis: G Orilisi, FV, VN, CS. Investigation: G Orilisi, FV, VT. Methodology: G Orilisi, FV, VN, CS. Project administration: GM, G Orsini, VQ. Resources: VT, SS, VQ. Software: VN, CS, VT, SS. Supervision: GM, EG, G Orsini. Validation: GM, EG, G Orsini, VQ. Visualization: VN, CS, VT, SS. Writing—original draft: G Orilisi, FV. Writing—review & editing: All authors.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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SUPPLEMENTARY MATERIAL

Supplementary data is available at <https://doi.org/10.4041/kjod25.296>

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