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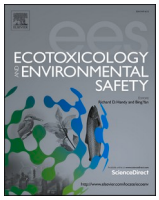

Type-specific inflammatory responses of vascular cells activated by interaction with virgin and aged microplastics

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ABSTRACT

Microplastics (MPs) are recognized as a major environmental problem due to their ubiquitous presence in ecosystems and bioaccumulation in food chains. Not only humans are continuously exposed to these pollutants through ingestion and inhalation, but recent findings suggest they may trigger vascular inflammation and potentially worsen the clinical conditions of cardiovascular patients. Here we combine headspace analysis by needle trap microextraction-gas chromatography-mass spectrometry (HS-NTME-GC-MS) and biological assays to evaluate the effects of polystyrene, high- and low-density polyethylene MPs on phenotype, metabolic activity, and pro-inflammatory status of Vascular Smooth Muscle Cells (VSMCs) the most prominent cells in vascular walls. Virgin and artificially aged MPs (4 weeks at 40 °C and 750 W/m^2 simulated solar irradiation) were comparatively tested at 1 mg/mL to simulate a realistic exposure scenario. Our results clearly show the activation of oxidative stress and inflammatory processes when VSMCs were cultured with aged polymers, with significant overexpression of IL-6 and TNF-α. In addition, volatile organic compounds (VOCs), including pentane, acrolein, propanal, and hexanal as the main components, were released by VSMCs into the headspace. Type-specific VOC response profiles were induced on vascular cells from different MPs.

1. Introduction

In recent years, microplastics (MPs), polymer particles conventionally including those below 5 mm, have attracted considerable attention as potential threats to ecosystems and human health, in connection with the alarming scientific pieces of evidence of massive and steadily increasing environmental pollution by plastic waste and debris in the whole size range, from large items down to nanoplastics (NPs, polymer particles in the sub-micrometer range) (Akdogan and Guven, 2019). Among the various plastics, polyolefins (high-density polyethylene, HDPE; low-density polyethylene, LDPE; polypropylene, PP) represent the largest share of newly produced polymers and arguably of the generated waste (Prata et al., 2019). They are commonly used for industrial, consumer goods, and food packaging applications (Hesler et al., 2019). Furthermore, polyethylene (PE) and polystyrene (PS) have been recently detected in amniotic fluid, placenta, and blood (Halfar et al., 2023; Braun et al., 2021,) and this underlies their capability to overcome human external barriers. PE and polyvinyl chloride (PVC) were recently found in carotid artery plaques from patients undergoing endarterectomy and strongly correlated with the risk of cardiovascular adverse events (Ragusa et al., 2021). Despite the often overemphasized persistence, it is well known that polymers are prone to several degradation processes (e.g., photo- and thermo-oxidation, hydrolysis) altering their mechanical, physical and chemical properties, and resulting in the

Abbreviations: HS, Head Space; HDPE, High-Density Polyethylene; HCASMCs, Human Coronary Artery Smooth Muscle Cells; IL-6, Interleukin 6; LDPE, Low-Density Polyethylene; MNPs, Micro/nano plastics; MPs, Microplastics; NPs, Nanoplastics; NTME-GC-MS, Needle Trap MicroExtraction-Gas Chromatography-Mass Spectrometry; NTDs, Needle Trap Devices; PS, Polystyrene; PE, Polyethylene; PP, Polypropylene; PVC, Polyvinyl chloride; PCA, Principal Component Analysis; ROS, Reactive Oxygen Species; TNF-α, Tumour Necrosis Factor-alpha; VSMCs, Vascular Smooth Muscle Cells; VOCs, Volatile Organic Compounds.

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above-mentioned generation of progressively smaller plastics fragments (Du et al., 2021). Such polymer degradation pathways play a significant role in determining the distribution of MPs in the ecosystems, in promoting or hampering the interaction with living organisms, and as well as in modulating their role as carriers of microorganisms and concentrators of bioaccumulable chemical pollutants such as plastics additives (plasticizers, antioxidants, metal oxides, etc.) and toxic substances captured from polluted environments (heavy metals, persistent organic compounds, pharmaceuticals, etc.) (Emenike et al., 2023; Yang et al., 2022; X. Wu et al., 2019). In addition, MPs are direct sources of potentially harmful low molecular weight products generated by polymer degradation (La Nasa et al., 2021; Lomonaco et al., 2020).

Due to the ubiquitous presence of MPs, most living organisms, including humans, are inevitably exposed through ingestion or inhalation (Wright and Kelly, 2017). In particular, the smaller sized ones can then be internalized by crossing the digestive tract or respiratory system walls, thus entering the circulatory system (Leslie et al., 2022; Persiani et al., 2023). Upon reaching different organs and tissues, starting from the vascular epithelium and including very sensitive ones such as, e.g. the placenta (Ragusa et al., 2021), they may accumulate, increasing the dose-related risks. However, data on the effects of MPs on humans and, more generally, on mammalian cells and tissues are still relatively scarce (Barcelò et al., 2023; Grote et al., 2023).

Some studies have documented different degrees of toxicological or pathological effects of MPs and NPs in fishes (Eliso et al., 2024; Zitouni et al., 2022) as well as in murine models (Deng et al., 2017; Li et al., 2020; Yang et al., 2019), suggesting the occurrence of oxidative stress and inflammation processes (Qiao et al., 2019). For example, MPs may alter the antioxidant system capacity of marine jacopever, leading to excessive production of reactive oxygen species (ROS) (Yin et al., 2018) and causing inflammation, apoptosis, and metabolic disorders in targeted tissues (Yu et al., 2020). ROS production causes multiple effects, including the reduced bioavailability of nitric oxide (NO) in the vascular endothelium and the increased blood levels of peroxy-nitrite. Both these effects may damage DNA, proteins, and lipids in the cells and generate cytotoxic effects (Gonzàlez-Acedo et al., 2021). Concerning the evaluation of the cellular response, the analysis of volatile organic compounds (VOCs) emitted from culture systems has recently become an important tool for non-destructive monitoring of cell proliferation and metabolism (Bischoff et al., 2018; Little et al., 2020; Traxler et al., 2019). Some VOCs (e.g., ethane, pentane, and hexanal) have been suggested as end-products of lipid peroxidation caused by ROS in the cells (Calenic et al., 2015), and thus as markers for the identification of various diseases (Sharma et., 2023).

Concerning the potential impact on human health, the induced oxidative stress and inflammation may trigger the activation of a plethora of pro-inflammatory mediators, culminating with the release of cytokines such as interleukin 6 (IL-6), interleukin-1β and tumour necrosis factor α (TNF-α) from human peripheral blood mononuclear cells (PBMCs), and histamine from mast cell lines (Chen et., 2023; Gonzàlez-Acedo et al., 2021; Hwang et al., 2019), all of the above being closely associated with chronic diseases such as cardiovascular diseases (Camps and Garcìa-Heredia, 2014), the leading causes of death and disability in the modern world (Vaduganathan et al., 2022).

Through *in-vitro* tests with PS NPs as models for polymeric drug carriers, Barshtein (Barshtein et al., 2016) found that the NPs induce deregulation in aggregation and endothelial adhesion of red blood cells, which may cause circulatory disorders. Moreover, MPs have been discovered in human thrombi, further supporting a causative role in cardiovascular diseases (Wu et al., 2023). These findings suggest a direct link between MPs internalization and the onset of the atherosclerotic process. Vascular Smooth Muscle Cells (VSMCs) are the main components of medium and large arteries and are involved in the pathophysiology of atherosclerosis. They display high plasticity in response to vascular damage and inflammation through phenotypic switching and participate in remodelling arterial walls. The VSMCs' transition into an activated phenotype induces a pro-inflammatory state, playing a pivotal role in the onset of atherosclerosis (Hu et al., 2019). Given the high incidence of cardiovascular diseases as the main causes of human death and disability and the growing evidence of the presence of MPs in human circulatory systems as a result of the increasing level of global pollution by MPs, it is crucial to clarify MPs' potentially toxic cardiovascular effects.

Currently, most studies use commercially available polymer particles, such as PS micro- and nanospheres. However, these particles do not truly represent real environmental micro-nanoplastics (MNPs) because they do not accurately replicate the complexity of surface chemistry, particle morphology and molecular release profile (Weber et al., 2022). Besides, as discussed before, studying the effects of MNPs on cells and tissues in vitro should be based not only on realistic particles but also on a multifactorial approach to evaluate the actual causal factor for the cellular response, which might be stimulated by one or more of the above particle features (size, polymer, surface chemistry, leached out species).

In the present study, we evaluated, for the first time, the effects of three common plastics, namely polystyrene (PS), high-density polyethylene (HDPE) and low-density polyethylene (LDPE) at the micro level, on VSMCs using aged materials that better simulate the real environmental samples. Specifically, we investigated cell viability and oxidative and inflammatory damage by monitoring Caspase-1, TNF-α, IL-6, and a panel of volatile organic compounds released from stressed VSMCs. The selected polymer types, HDPE, LDPE, and PS, are among the most common hydrocarbon polymers found as environmental micropollutants. As a further innovative approach, we combined headspace analysis by needle trap microextraction-gas chromatography-mass spectrometry (HS-NTME-GC-MS) with biological assays to evaluate the effects of virgin and artificially aged MPs on phenotype, metabolic activity, and pro-inflammatory status of VSMCs.

2. Materials and methods

2.1. Microplastics features

Cryogenically micronized powders of poly(styrene) (PS) (nominal density of 1.05 g/cm^3 and an average particle size of 564 μm), high-density poly(ethylene) (HDPE) (density of 0.952 g/cm^3 and an average particle size of 622 μm) and low-density poly(ethylene) (LDPE) (density of 0.917 g/cm^3 and an average particle size of 632 μm) were a kind gift from Poliplast S.p.A. (Casnigo, Italy). These virgin materials, named from now onwards "0_W", were artificially aged in a solar box with an Xenon lamp to accelerate their photo-oxidative degradation according to the procedure described elsewhere (Lomonaco et al., 2020). Aged materials were named "4_W" here. Samples were stored at −20°C in amber glass vials until their use. Before each use, they were allowed to equilibrate at room temperature and sterilized with three cycles of 15 mins under UV light. The chemical characterization of both virgin and aged materials is reported elsewhere (Castelvetro et al., 2021).

Working suspensions of polymers were prepared in culture medium (supplemented Medium 231, Thermo Fisher, Waltham, MA, USA) at 1 mg/mL concentration, considered an environmental exposure concentration as suggested (Wang et al., 2021).

2.2. Cell culture and experimental plan

Human Coronary Artery Smooth Muscle Cells (HCASMCs, Thermo Fisher, Waltham, MA, USA) were used to conduct the presented experiments. Throughout the text, we refer to this cell type as VSMCs (Vascular Smooth Muscle Cells). VSMCs were subcultured at 37 °C and 5 % of CO_2 employing a humidified cell culture incubator in either 25 cm^2 culture flasks (for exhaled VOCs analysis) or 96-multiwell plates (for cell viability, oxidative stress, and inflammation assay), containing

Medium 231 supplemented with Smooth Muscle Growth Supplement (SMGS, Thermo Fisher, Waltham, MA, USA) and 1 % antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin). For VOCs analysis, the flasks were closed with a lab-made cap equipped with a polycarbonate male luer-lock to thread adapter (10−32) (Supelco, Italy), a polycarbonate three-way stopcock (Supelco, Italy), and a male luer-lock IN-Stopper (Braun, USA) enabling the insertion of commercial side-hole needle trap devices (NTDs). Cells were allowed to settle in supplemented Medium 231 for 24 hours and then incubated for 3 and 7 days with 1 mg/mL virgin or aged plastic materials. Culture media were replaced every 48–72 hours. VSMCs were used for *in vitro* reactive oxygen species (ROS) and caspase-1 activity measurements. For a 96-multiwell plate format, VSMCs were seeded at 2000 per well for ROS quantification or 6000 per well for Caspase-1 detection after 3 days, and roughly 1700 or 4000 per well, respectively, when analysed after 7 days.

2.3. Cell viability assay

The metabolic capacity of VSMCs was assessed through the CellTiter-Blue® Cell Viability Assay kit (Promega, Madison, WI, USA). Briefly, viable and healthy cells reduce resazurin to resorufin, generating a fluorescence signal proportional to the number of cells. VSMCs were analysed 3 and 7 days after adding virgin and aged materials. To perform the VSMC viability assay, 20 μL of CellTiter-Blue® Reagent was used for each 100 μL of the medium in a 96-multiwell. The mixture was added to each sample and then incubated at 37°C for at least one hour to allow the reaction to occur. Fluorescence was measured at $560_{Ex}/590_{Em}$ nm wavelengths using a Fluostar Omega spectrofluorometer (BMG Labtech, Ortenberg, Germany).

2.4. Oxidative stress measurement

Intracellular ROS induced in VSMCs by plastic exposure was assessed with ROS-Glo™ H_2O_2 Assay kit (Promega, Madison, WI, USA). Samples were taken at 3 and 7 days post-exposure and then processed according to the manufacturer's instructions. Briefly, 20 μL/well of H_2O_2 substrate solution was added to each sample to be analysed and then incubated for 3 hours at 37 °C. Then, 50 μL/well of cell medium was transferred in a 96-white multiwell plate mixing with 50 μL/well ROS-Glo™ Detection Solution. After a 20 mins-incubation at room temperature and protected from light, relative luminescence units (RLU) of the entire spectra were measured using a plate reader.

2.5. Inflammasome and inflammatory markers

The toxicity of MNPs in cells may trigger the induction of inflammasome complexes in several tissues, enabling the activation of Caspase-1-dependent signalling pathways generated by inflammatory stimuli resulting from oxidative stress (Banerjee and Shelver, 2020). To test Caspase-1 induction, the Caspase-Glo® 1 Inflammasome Assay kit (Promega, Madison, WI, USA) was employed according to the manufacturer's instructions. In summary, all reconstituted reagents were allowed to equilibrate at room temperature. An aliquot (20 μL) of cell medium from each 96-multiwell plate was transferred to a 96-white multiwell plate. Then, samples were treated with 50 μL/well of Caspase-Glo® 1 Reagent (for the detection of Caspase-1) or 50 μL/well of Caspase-Glo® 1 YVAD-CHO Reagent (as an inhibitor to exclude any non-specific effects). Samples were incubated for 1 hour at room temperature and protected from light. Relative luminescence units (RLU) were measured using a plate reader.

IL-6 was measured in VSMCs media upon 3 and 7 days of exposure through automated ELISA (Elecsys® IL-6, Roche Diagnostic, Basel, Switzerland). The Roche Elecsys IL-6 assay is a non-competitive chemiluminescent immunoassay. An aliquot (30 μL) of each sample was first incubated with anti-IL-6 antibodies, followed by a second incubation with anti-IL-6 antibodies labelled with ruthenium. The resulting complexes were magnetically captured, and a chemiluminescent emission signal was generated, directly proportional to the IL-6 concentration present in the media. The limit of detection was defined to be 1.5 pg/mL.

For TNF-α detection, TNF-α Human ELISA Kit (Thermo Fisher, Waltham, Massachusetts, USA) was used, following the manufacturer's instructions. Briefly, 50 μL of cell supernatant was incubated in a 96-well plate containing immobilised monoclonal TNF-α antibodies. Afterwards, the biotin-conjugated anti–TNF-α and streptavidin–horseradish peroxidase were added to each well with intermediate washing steps. After that, the antibody–protein complex was detected by adding the chromogenic substrate (tetramethylbenzidine), and absorbance was measured at 450 nm. The sensitivity of human TNF-α, defined as the concentration resulting in an absorbance significantly higher than that of the diluent, was determined to be 0.13 pg/mL.

2.6. Headspace sampling and analysis

Before first use, NTDs were conditioned for 15 h at 300°C using a custom-made heating device (PAS-Technology, Germany) applying 1 bar of nitrogen 5.0 (Sol Group Spa, Italy). Right before use, NTDs were conditioned again for 30 min using the same procedure.

An aliquot (25 mL) of head space fraction (total volume of 50 mL) was transferred into commercial side-hole NTDs (23-gauge stainless steel needle, length 60 mm) (PAS-Technology, Germany), packed with 3 cm of Tenax GR (60/80 mesh) at 5 mL/min using a sampling device enabling to set sampling volume (mL) and flow rate (mL/min) (Biagini et al., 2019). After sampling, NTDs were immediately sealed with Teflon caps and stored at room conditions (T = 25 °C and relative humidity 60 %) to preserve the sample composition (Biagini et al., 2019).

Needle trap devices were automatically desorbed for 20 s at 300 °C in the gas-chromatograph inlet using an autosampler (PAS-Technology, Germany). Compounds were analysed with a 7890B GC (Agilent Technologies, USA) coupled with a 7010 triple quadrupole mass spectrometer (Agilent Technologies, USA). The chromatographic separation was carried out on a DB-624 ultra-inert capillary column (60 m × 0.25 mm, 1.4 μm film thickness) from Agilent Technologies (USA). Helium 5.6 (split flow of 5 mL/min) was used as a carrier gas at a constant flow of 1 mL/min. The oven temperature program was: 35 °C for 15 min, 4 °C/min up to 130 °C (3 min hold time), and 10 °C/min up to 220 °C (1 min hold time). Post run was 15 min with an oven temperature of 250 °C and a Helium flow rate of 1.5 mL/min. The mass spectrometer was operated in a full scan mode, with an m/z range set from 30 to 200, and in selected ion monitoring. Quantifier (Q) and qualifier (q) ions were monitored with a constant dwell time of 50 ms. The transfer line, ion source, and quadrupoles operated at 260, 250, and 150 °C, respectively. Chromatographic data were acquired using MassHunter software (v. B.07.00, Agilent Technologies, USA).

2.7. Data processing and statistical analysis

All tests were normalised for the cell number in the sample by creating a dilution curve. A defined number of VSMCs serially diluted was seeded in the desired plate/flask format, then they were allowed to settle for 16–24 hours, and cell viability was measured as previously described. Thus, a standard curve was created, linking the fluorescent value (cell viability) with the relative number of cells. The template generated was applied to obtain the cell number in each experiment.

Gas chromatography–mass spectrometry data were filtered off when i) the peak area of target analytes in the head space fraction was about a half of that found in room air samples and ii) there was the presence of missing data in at least 50 % of the samples.

The presence of target VOCs (Table S1) in the Supplementary Material) in the headspace fraction of cell samples was confirmed by comparing the actual retention time and qualifier/quantifier (q/Q) ratio to the analysis of a standard mixture. The maximum variation of

retention time and q/Q ratio was fixed at ±0.10 min and 10 %. The final data matrix containing both VOCs and biological data was analysed by R software (4.1 version).

Missing (i.e., values below the detection limit) data were replaced with a random value between 0 (excluded) and the minimum positive value of each variable. A decimal logarithmic transform was used to correct for asymmetry characterising all the variables (Oliveri and Forina, 2012). Normality and homogeneity of variance were tested using the Shapiro-Wilk and Levene tests, respectively. Data were analysed using a multivariate exploratory method (principal component analysis, PCA). PCA was applied after column autoscaling to ensure the same importance a priori to be given to all variables, irrespective of their magnitude (Jolliffe, 2002).

Continuous variables with a normal and a skewed distribution were reported as mean ± standard deviation (SD). ANOVA and Kruskal-Wallis tests were used for multiple comparison. Bonferroni test was used for multiple pairwise comparisons. The relationship between variables was examined by Pearson's and Spearman's correlation. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Effects of virgin and aged PS-, HDPE-, and LDPE-MPs on VSMC viability

Fig. 1 shows the effects of incubation of virgin and aged MPs on VSMCs.

When MPs were compared to the control (CTRL), no significant changes were observed after 3 days of exposure, except for virgin HDPE-MPs (HDPE_0 W) that reduced cell viability (-25 %) compared to the control group and aged PS-MPs (PS_4 W). After 7 days of exposure, the cell viability of virgin HDPE (HDPE_0W) and aged HDPE (HDPE_4W), virgin LDPE (LDPE_0W) and aged PS (PS_4W) changed significantly compared to control (CTRL) samples.

Compared to 3 days of exposure, a statistically significant increase in cell viability was noticed for control (CTRL, 10400 ± 180 *vs.* 14280 ± 260, $p<0.01$), for aged LDPE (LDPE_4W, 10100 ± 120 *vs.* 13800 ± 830, $p<0.05$), and for aged PS (PS_4W, 11100 ± 100 *vs.* 14900 ± 90, $p<0.0001$), suggesting that the presence of MPs irrespective of their ageing, does not significantly affect cell viability occurred in these groups at the concentration tested.

3.2. Oxidative damage in VSMCs following exposure to PS-, HDPE-, and LDPE-MPs

To assess the MPs' cytotoxic potential, cellular oxidative stress in VSMCs was investigated by monitoring the amount of hydrogen peroxide (H_2O_2) as a representative ROS released in a culture medium upon treatment with virgin and aged MPs for 3 days and 7 days (Fig. 2).

A clear increase in ROS production was observed after the first 3 days of exposure to any one of the MPs except for virgin LDPE (LDPE_0 W), followed by a statistically significant attenuation within the 7 days of the experiments (Table S2).

In particular, after 3 days of exposure, both virgin and aged HDPE and PS induced a nearly three- and two-fold increase in ROS levels compared to control samples, respectively. A similar increase (two times) was also observed for aged LDPE. After 7 days, the oxidative stress levels were lower than those observed after 3 days (Table S2) and

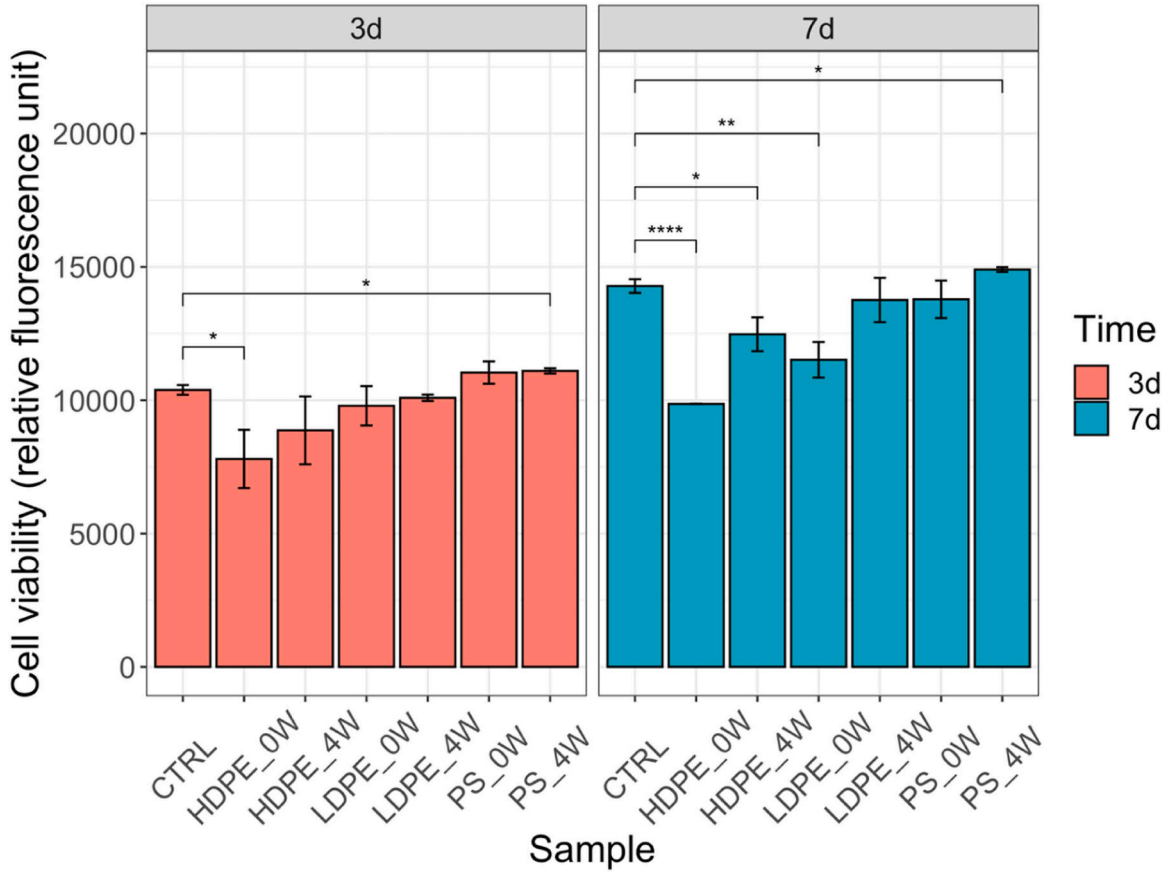

Fig. 1. Comparison between cell viability in control (CTRL) and VSMC samples treated with different virgin (0 W) and aged (4 W) MPs (HDPE: high-density polyethylene, LDPE: low-density polyethylene, and PS: polystyrene) at 1 mg/mL for 3 days (3d) and 7 days (7d). All t-tests (two-tailed) were corrected for multiple comparisons using Bonferroni correction. $n = 2$ biologically independent experiments. Legend: *$p<0.05$, **$p<0.01$, ****$p<0.0001$.

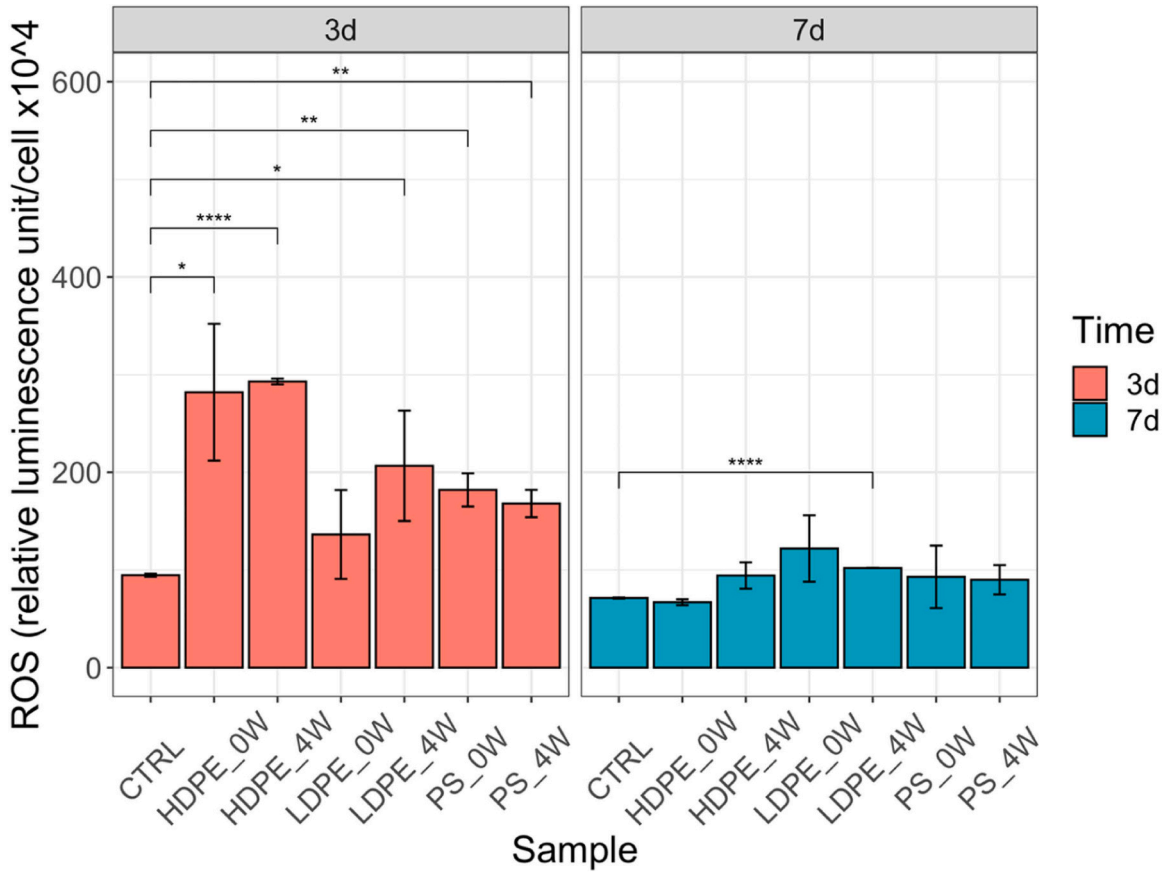

Fig. 2. Comparison between ROS in control (CTRL) and VSMC samples treated with different virgin (0 W) and aged (4 W) MPs (HDPE: high-density polyethylene, LDPE: low-density polyethylene, and PS: polystyrene) at 1 mg/mL for 3 days (3d) and 7 days (7d). All t-tests (two-tailed) were corrected for multiple comparisons using Bonferroni correction. $n = 2$ biologically independent experiments. Legend: $^{*}p<0.05$, $^{**}p<0.01$, $^{****}p<0.0001$.

not statistically different from control samples, except for aged LDPE with higher ROS levels (+25 %).

3.3. *Effects of exposure to virgin and aged PS-, HDPE-, and LDPE-MPs on the inflammatory factor expressions*

To explore the MPs' potential to elicit an inflammatory response in VSMCs, the concentration levels of a panel of molecular markers of inflammation (i.e., Caspase-1, IL-6, and TNF-α) were measured. A sustained and significant up-regulation of caspase-1 levels was observed in all the samples at 3 days, except for aged HDPE (HDPE_4 W), but only for aged PS (PS_4W) after 7 days (Fig. 3).

Secretion of the pro-inflammatory cytokine IL-6 was evaluated in VSMCs upon stimulation with MPs. A generally significant increase in IL-6 levels was clearly evident when samples treated with MPs were compared to control samples after 3 days of incubation (Fig. 4). After 7 days, the release of IL-6 remains sustained for all the samples, with virgin HDPE (HDPE_0 W) and aged PS (PS_4 W) showing a significantly higher cell response when compared to the corresponding aged (16380 ± 1450 *vs.* 5240 ± 285, $p<0.05$ for HDPE_0W) and unaged MPS (10590 ± 1160 *vs.* 5240 ± 285, $p<0.05$ for PS_0W), respectively.

Similar results were observed for TNF-α, an important player in the inflammatory cascade. Compared to control samples, a statistically significant increase in TNF-α values was observed regardless of MPs after 3 days, and the same difference was also evident after 7 days (Fig. 5).

3.4. *VOCs changes in VSMCs exposed to virgin and aged PS-, HDPE-, and LDPE-MPs*

Headspace (HS) analysis by Needle Trap Micro Extraction (NTME) coupled to gas chromatography–mass spectrometry (GC-MS) was performed to gain insights into the effects of the volatile chemicals released from virgin and aged PS-, HDPE-, LDPE- MPs on VSMCs. The experimental variability of the HS-NTME-GC-MS protocol ranged between 20 % and 30 %, probably due to the several steps needed to manipulate cells, as discussed elsewhere (Kü et al., 2016). VOCs showing a signal close to the instrumental limit of detection (IDL) or a concentration value comparable (±20 %) to the culture media background, and signals from linear and cyclic siloxanes mainly due to the septa used for the analysis, were excluded from the statistical analysis. Ambient air contaminants (e.g., ethanol and isopropyl alcohol) were also excluded. Since MPs release VOCs during degradation (Lomonaco et al., 2020), blank samples of background culture media and MPs, respectively, were also incubated at 37 °C and 5 % CO_2, and analysed. The amounts of compounds emitted by such blank samples contributed marginally (<5 %) to the VOCs profile typically observed when VSMCs were combined in the culture media with the different types of virgin and aged MPs. Based on these criteria and observations, we selected 28 VOCs for further statistical analyses (Table S1). Most VOCs were ketones (12 out of 28), aldehydes (8 out of 28), and alkanes (5 out of 28). The remaining three VOCs were carbon sulphide, ethyl acetate, and 2-methyl-1-propanol. Fig. 6 shows the results of principal component analysis (PCA) carried out on the selected VOCs.

The PCA score plot revealed a type-specific metabolic profile for VSMCs treated with MPs (Fig. 6A). The first two principal components (PC1 and PC2) explained about 55 % of the variance, effectively separating control cells from VSMCs treated with MPs. Compared to controls, cells incubated with MPs showed a significant increase of ROS, TNF-α, and IL-6, clearly indicating the activation of oxidative stress and inflammation processes. Interestingly, most VOCs that are mainly

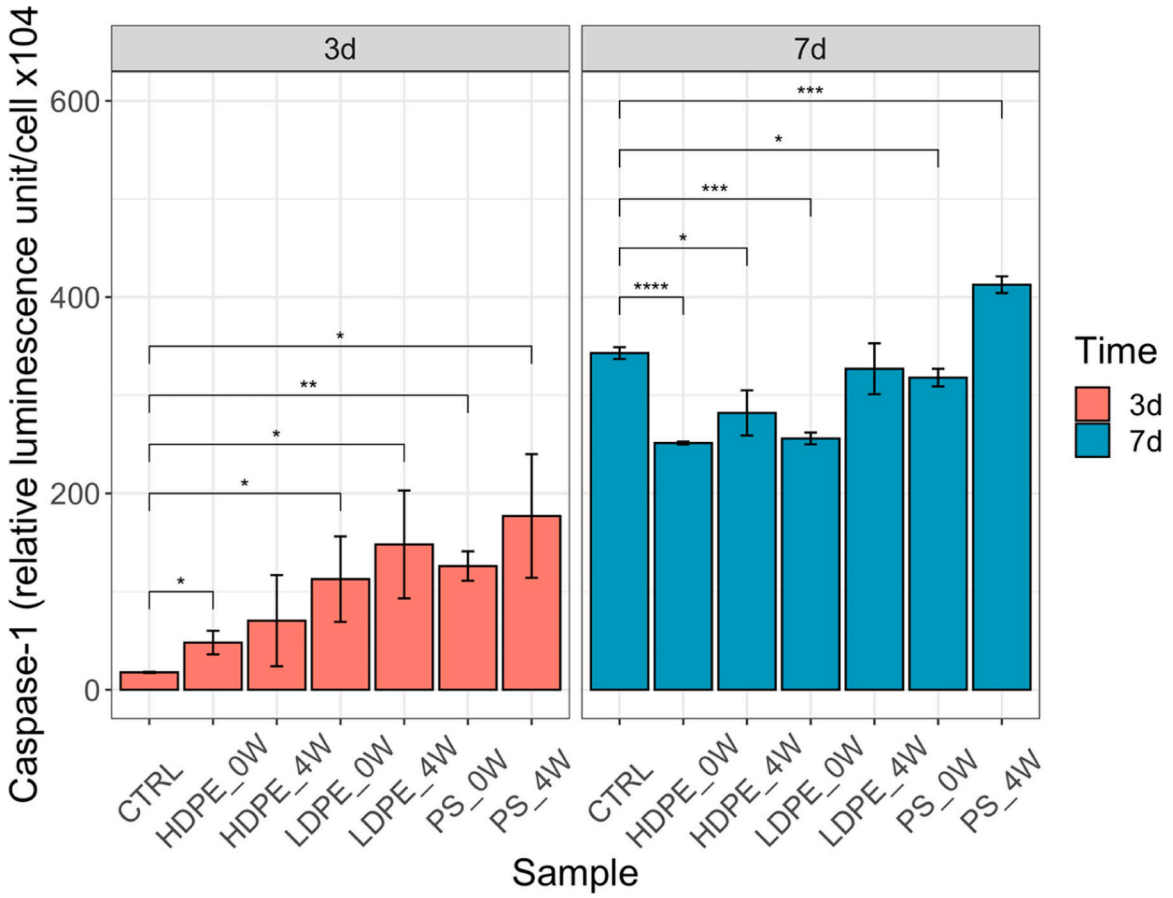

Fig. 3. Comparison between Caspase-1 in control (CTRL) and VSMC samples treated with different virgin (0 W) and aged (4 W) MPs (HDPE: high-density polyethylene, LDPE: low-density polyethylene, and PS: polystyrene) at 1 mg/mL for 3 days (3d) and 7 days (7d). All t-tests (two-tailed) were corrected for multiple comparisons using Bonferroni correction. $n = 2$ biologically independent experiments. Legend: *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

contributing (>10 %) to PC2 (Fig. 6B), e.g., acrolein, pentane, propanal, 2-methyl pentane, and hexanal, were also overexpressed, suggesting a potential relationship between these altered pathways and the cited VOCs. An effect of incubation time (i.e., from 3 days to 7 days) on the overall VOCs profile was mainly observed for virgin MPs and only to a lower extent for aged materials, as highlighted by the decrease of most VOCs contributing to PC1 (Fig. 6). After 7 days of incubation, cells treated with virgin HDPE-MPs showed a marked reduction (30–50 %) of variables contributing to PC1, such as benzaldehyde, 2,3-butanedione, carbon disulphide, and 2-methylbutanal, suggesting a potential consumption or binding of these VOCs by cells, as recently discussed for benzaldehyde by Klemenz et al. (Klemenz et al., 2019). Cells treated with virgin PS-MPs showed the opposite behaviour, mainly due to the increase of 5-methyl-3-heptanone, 3-octanone, and 2-pentanone.

Fig. 7 shows differences between control and VSMC samples treated with virgin and aged MPs, for pentane, acrolein, propanal, 2-methyl pentane, and hexanal.

The presence of aged MPs induced the production of pentane, acrolein, propanal, and hexanal after 3 days of exposure. Interestingly, when exposure lasted 7 days, the concentrations of these VOCs decreased (Table 1) while remaining significantly higher than in control samples, showing trends similar to those found for ROS (Fig. 2). Exposure to virgin MPs caused less marked effects without a clear and univocal trend.

4. Discussion

While MPs' effects on human health are still unclear, preliminary animal studies highlighted their ability to induce oxidative stress and inflammation (Qiao et al., 2019). Moreover, synthetic polymers stimulate the over-production of pro-inflammatory cytokines and promote haemolysis and a pro-thrombotic state (Hwang et al., 2019). Concerning the cardiovascular system, the experimental evidence collected so far and summarized in the introduction suggests that MPs may contribute to triggering vascular inflammation and symptoms of cardiovascular diseases, potentially worsening the clinical conditions of cardiovascular patients (Barshtein et al., 2016; Zhu et al., 2023).

Building on our previous results highlighting that artificially aged MPs release potentially harmful volatile molecular species (VOCs), enhancing their toxicity (Lomonaco et al., 2020), in the present study, we started investigating the effects of MPs on human cells. In particular, we combined an advanced analytical protocol for HS analysis of the VOCs with biological assays to understand their effects on VSMCs, the latter serving as model cells of the cardiovascular system.

VSMCs were selected for the experiments presented here based on the assumption that MPs cause vascular damages with an inflammatory component, as in the case of atherosclerosis. VSMCs aberrant proliferation favours pathological plaque formation, therefore promoting atherosclerosis (Klemenz et al., 2019). VSMCs isolated from human coronary arteries are an excellent *in vitro* model for directly exploring molecular inducers of cardiovascular pathologies like atherosclerosis and their relationship with endogenous and exogenous players, such as MPs.

Concerning the VOCs analysis, their efficient extraction at trace levels using a limited sample volume (e.g., tens of millilitres) is one of the main advantages of the NTME approach over the common active sorbent phase extraction sampling (Lord et al., 2010). The lab-made cap guaranteed a tight flask closure, preventing sample leaks during the HS

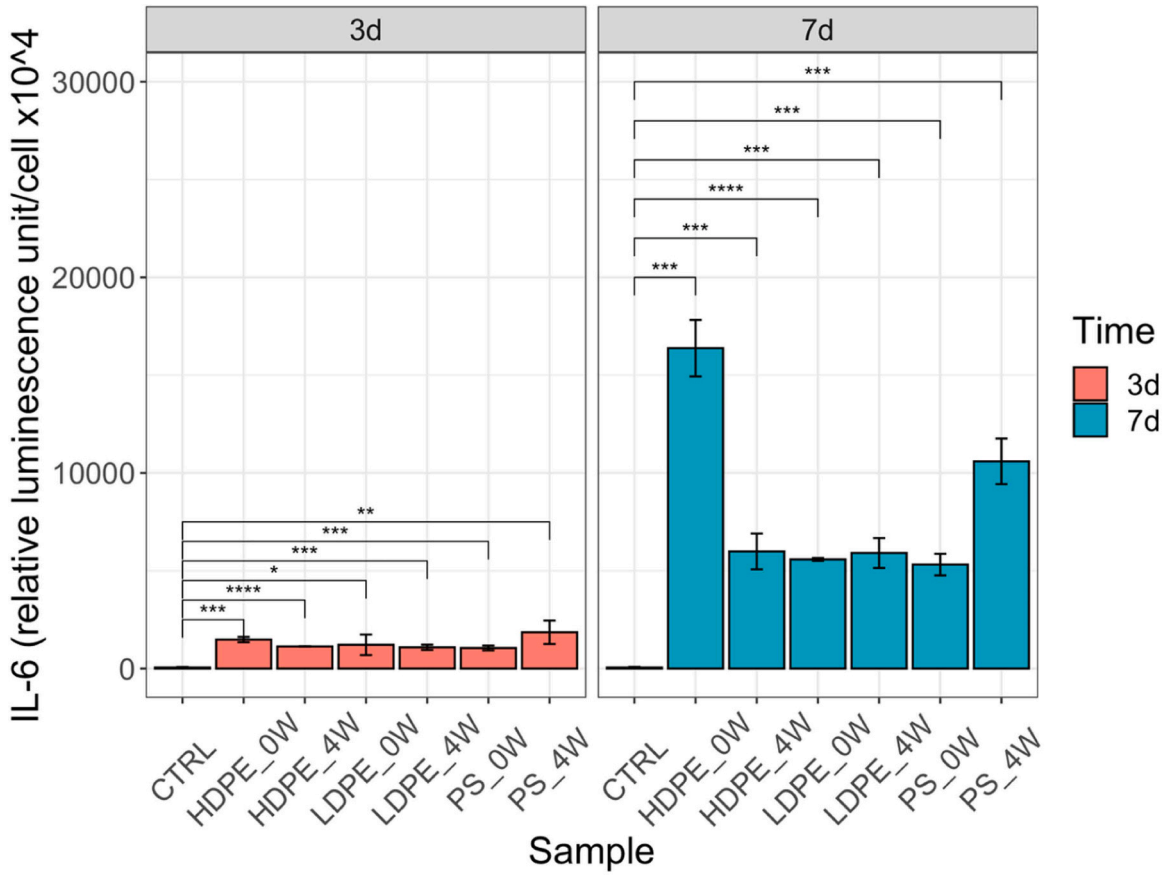

Fig. 4. Comparison between IL-6 in control (CTRL) and VSMC samples treated with different virgin (0 W) and aged (4 W) MPs (HDPE: high-density polyethylene, LDPE: low-density polyethylene, and PS: polystyrene) at 1 mg/mL for 3 days (3d) and 7 days (7d). All t-tests (two-tailed) were corrected for multiple comparisons using Bonferroni correction. $n = 2$ biologically independent experiments. Legend: $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{***}p<0.0001$.

collection. The experimental setup showed a limited background emission, mainly from linear and cyclic siloxanes. The analysis of samples immediately after the removal of cells from the incubator (set at 37°C) combined with the reduced sampling time (5 minutes) required for the HS sampling minimised the cooling of the flask, avoiding the water condensation on its walls that represents a major problem for HS analysis with sorbent materials (Dettmer and Engewald, 2002). Twenty-eight VOCs, belonging to alkanes, aldehydes, ketones, and alcohols (Table S1), were successfully monitored through the HS-NTME-GC-MS approach, allowing assessment of the different response of VSMCs when treated with virgin and aged MPs. Interestingly, VSMCs treated with PS, HDPE, and LDPE MPs showed peculiar VOCs profiles, as highlighted by the PCA analysis (Fig. 6).

Specific correlations were observed among biological parameters and some VOCs. Propanal moderately correlated with cell viability ($r = 0.37$, $p = 0.03$). Muzio (Muzio et al., 2012) showed how propanal, together with other aldehydes and alcohols, may be derived from propionyl-CoA through the activity of dehydrogenase, which is characteristic of cell proliferation. Although partially affecting cell viability, MPs did not stop cell growth (Fig. 1).

Regarding the connection between VOCs and the inflammatory state, we observed that PS, HDPE, and LDPE MPs triggered one or more components of the inflammasome complex, as highlighted by the increase of Caspase-1 and pro-inflammatory cytokines (i.e., IL-6 and TNF-α) (Figs. 3–5). A plethora of VOCs can be generated under oxidative stress and inflammation conditions (Aranda-Rivera et al., 2022). In particular, ROS induce the peroxidation of lipids and other biomolecules (Endale et al., 2023; Trefz et al., 2013), causing the release of several alkanes, including pentane. In our samples, a moderate correlation ($r = 0.31$, $p = 0.04$) was observed between pentane and ROS, confirming a potential link between these two variables when VSMCs are treated with MPs. Moreover, particular modulation of acrolein has been associated with inflammation. Acrolein is an α,β-unsaturated aldehyde with an irritating action on the respiratory system that, at high concentrations, has cytotoxic effects on many cell types, including hepatocytes, neurocytes, and alveolar epithelial cells (Mohammad et al., 2012). Furthermore, acrolein can induce an inflammatory response in macrophages (Yang et al., 2013) and epithelial cells (Dwivedi et al., 2018), which is strongly associated with cardiovascular risk (DeJarnett et al., 2014). Our results confirm that acrolein is associated with inflammation markers such as IL-6 ($r = 0.45$, $p = 0.047$) and TNF-α ($r = 0.50$, $p = 0.01$).

Virgin and aged HDPE-MPs, as well as virgin LDPE-MPs, reduced cell viability compared to the control (Fig. 1). This phenomenon had already been observed in previous studies (Dong et al., 2020; Hesler et al., 2019; Stock et al., 2020), but researchers were not able to propose a possible explanation (Lehner et al., 2020; Schirinzi et al., 2017; Wu et al., 2019). The cytotoxicity of MPs is frequently associated with membrane damage and oxidative stress (Ogale and Kamble, 2022). Generally, the alteration or disruption of membranes causes the generation of ROS in the mitochondria. These chemical species are responsible for mitochondrial damage by inducing the release of pro-apoptotic factors and the production of pro-inflammatory cytokines, which favour apoptosis.

Differences in the ROS levels in our samples (Fig. 2) suggest that VSMCs successfully used some defence mechanisms to counteract the harmful effects of some MPs.

Our results showed a significant increase of Caspase-1 levels (Fig. 3) in culture media treated with virgin and aged MPs at 3 days of incubation compared to controls, and less evident at 7 days with the

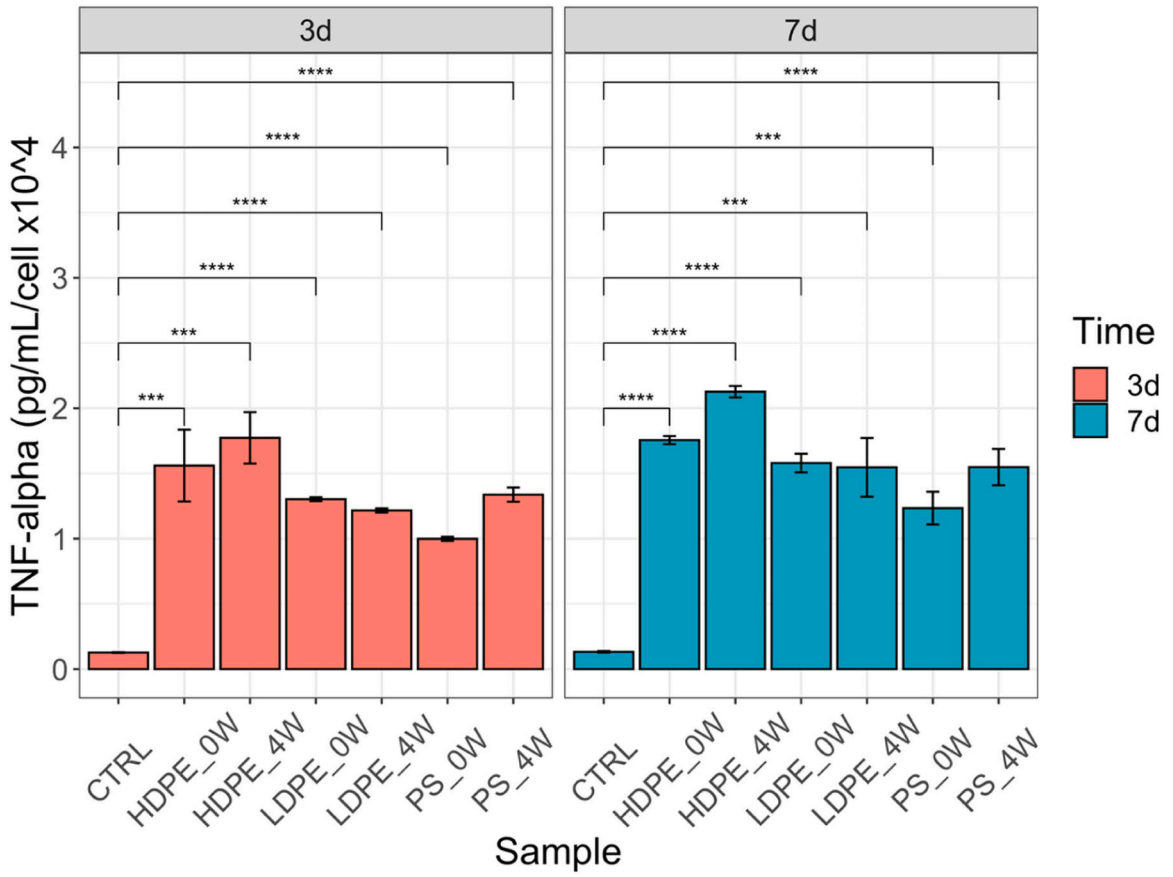

Fig. 5. Comparison between TNF-α in control (CTRL) and VSMC samples treated with different virgin (0 W) and aged (4 W) MPs (HDPE: high-density polyethylene, LDPE: low-density polyethylene, and PS: polystyrene) at 1 mg/mL for 3 days (3d) and 7 days (7d). All t-tests (two-tailed) were corrected for multiple comparisons using Bonferroni correction. $n = 2$ biologically independent experiments. Legend: $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

exception of aged PS (PS_4W). A possible explanation can be associated with a potential activation of apoptotic mechanisms (Molla et al., 2020) for the mid-term culture of cells, which reach a high confluence level and, consequently, promote apoptosis.

A clear indication of the effect of MPs on cultured cells is the overall pro-inflammatory state observed in our samples, as highlighted by the increased levels of IL-6 and TNF-α (Figs. 4–5). Under our experimental settings, the increase in TNF-α was independent of the specific MP type, as both virgin and aged MPs from all three polymers induced a comparable up-regulation. TNF-α is known to trigger several inflammatory molecules, including other cytokines (Banerjee and Shelver, 2020); therefore, the massive production revealed by the present study suggests a marked pro-inflammatory state.

The other relevant cytokine for evaluating an inflammatory response is IL-6, the most prominent muscle-derived protein produced in response to infections and tissue injuries, which stimulates the inflammasome complex. All tested MPs induced a marked IL-6 production after 3 days of exposure. Our results confirm previous studies of IL-6 and TNF-α production in monocytes and macrophage cell lines upon stimulation with PS or PP particles, irrespective of ageing (Hwang et al., 2019; Prietl et al., 2014). Increased levels of inflammatory biomarkers have recently been detected in *ex vivo* atheromatous plaque from patients, with a strong correlation of the presence of MPs strongly correlated with cardiovascular events (Marfella et al., 2024). The present results further substantiate and confirm such correlation with additional insights on the possibly contribution of molecular species deriving from the degrading MPs as causal co-factors of cardiovascular risk.

Overall, we can assume that depending on the polymer used, some difference on cell viability could only be observed between PE (high or low density) and PS more over longer contact times. As for the oxidative stress marker, results are more homogeneous between polymer types after 3 days of exposure. Inflammatory molecule activations tended to be comparable between MP groups, regardless of the time point considered.

Interestingly, our results highlighted the importance of using more realistic aged PS-MPs to assess the metabolic response. In fact, most studies use commercially available polymer particles, which do not truly represent real environmental micro- and nanoplastics because they do not accurately replicate the complexity of surface chemistry, particle morphology, and molecular release profiles. Compared to control samples, cells incubated with aged PS-MPs significantly upregulated the production of Caspase-1, IL-6, and TNF- α and emitted considerably larger amounts of acrolein, pentane, propanal, and hexanal (Fig. 7).

Many factors may contribute to the activation of the oxidative stress and inflammation responses observed with treated VSMCs. First, the aged polymers used in this study have altered surface properties due to artificial photo-oxidative ageing (with solar spectrum-simulating xenon lamp) that significantly produces oxidized groups, such as hydroxyl, hydroperoxyl, and carbonyl (aldehyde, ketones, carboxyl, and derived groups) (La Nasa et al., 2021; Lomonaco et al., 2020). Thus, the possible contact between aged MPs and cell membranes can be considered a cause of membrane damage and oxidative stress. Secondly, under our experimental conditions, upon ageing, the degraded polymers generate a number of more or less harmful degradation products (e.g., aromatic compounds for PS, aldehydes for HDPE and LDPE) (La Nasa et al., 2021; Lomonaco et al., 2020) that are mostly leached in the cell culture medium; these may then interact with VSMCs, triggering oxidative stress and inflammation processes. This aspect was confirmed by the analysis

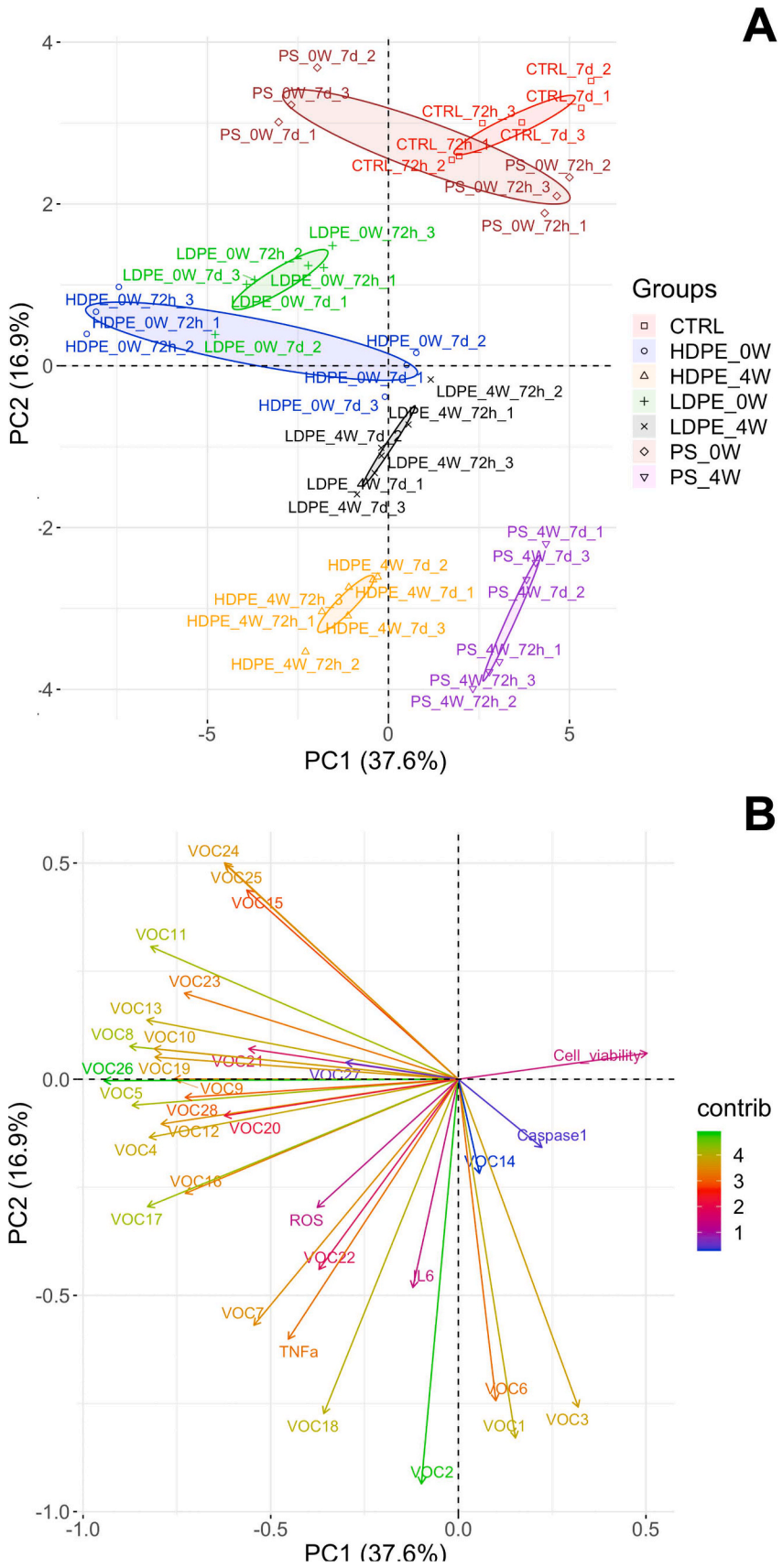

Fig. 6. Score plot **(A)** and loading plot **(B)** of the principal component analysis (PCA) performed on the data matrix containing VOCs and biological data obtained by the analysis of virgin (0 W) and aged (4 W) MPs (HDPE: high-density polyethylene, LDPE: low-density polyethylene, and PS: polystyrene) on VSMCs after 3 days (3d) and 7 days (7d) of incubation ($n = 3$ biologically independent experiments). The contribution of each variable to PCs is shown by the colour legend (blue low contribution, green high contribution) of the loading plot. The VOC IDs are reported in Table S1.

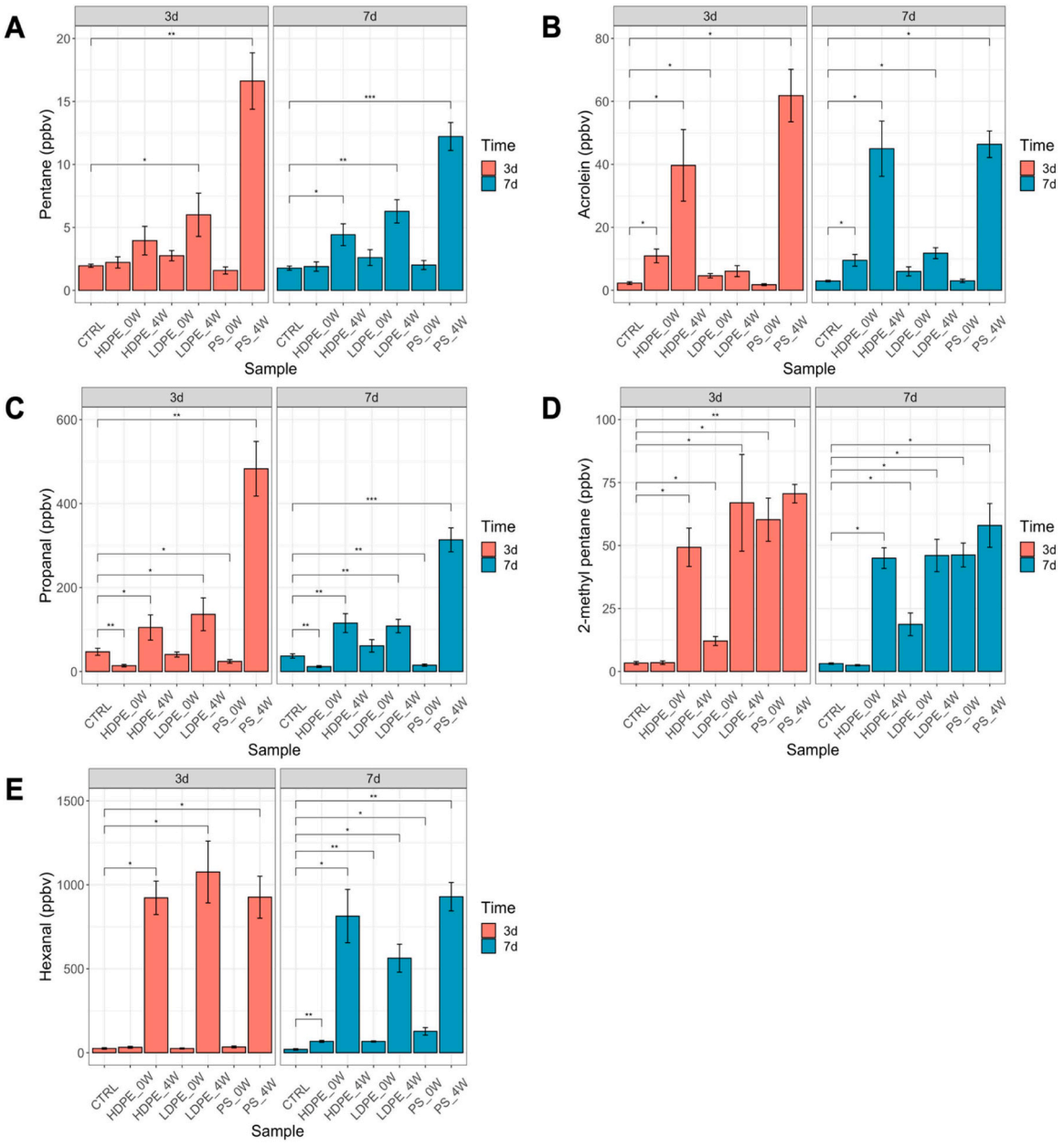

Fig. 7. Comparison between VOC levels in control (CTRL) and VSMC samples treated with different virgin (0 W) and aged (4 W) MPs (HDPE: high-density polyethylene, LDPE: low-density polyethylene, and PS: polystyrene) at 1 mg/mL for 3 days (3d) and 7 days (7d). All t-tests (two-tailed) were corrected for multiple comparisons using Bonferroni correction. $n = 3$ biologically independent experiments. Legend: $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

of sample media of aged MPs, where detectable amounts (roughly estimated at nanogram levels) of methyl vinyl ketone, methyl propenyl ketone, benzene, toluene, ethylbenzene, and benzaldehyde were observed. All these VOCs have been reported as reactive chemical species that may interact with the cells, inducing oxidative stress and an inflammatory status (Ogbodo et al., 2022). In the case of the vascular system, cells respond and adapt their physiology to variable conditions by modifying the vascular function and homeostasis (Prozialeck et al., 2008).

The inflammatory status is a constant of the entire set of the tested MPs, indicating that these materials could affect the cell behaviour over a long period. For example, VSMC proliferation and consequent migration are known to be triggered by inflammatory stimuli (Rastogi et al., 2012), which may also explain why cell survival was not compromised herein. Our experimental evidences further support, and demonstrates for a highly sensitive system (the VSMCs), that MPs may represent an emerging potential risk factor for cardiovascular disease since they exacerbate both oxidative stress and inflammation within the muscle cells of the aortic wall.

5. Study limitations

The present study describes the potentially harmful effects of MPs on VSMCs, one of the main components of the arterial wall, and suggests a possible physio-pathological role of MPs with a specific influence on cardiovascular diseases. Distinct contributions in cell biology perturbations have been highlighted for different types of MPs, namely virgin and artificially aged PS, HDPE and LDPE. Furthermore, this is the first study in which the VOCs released from VSMCs incubated with both virgin and aged MPs have been analysed. Through measurement of the VOCs, we were able to investigate the influence of each MP type (both virgin and in a more realistic state of degradation) on cells

Table 1
Comparison between control and VSMC samples treated with virgin (0 W) and aged (4 W) MPs (HDPE: high-density polyethylene, LDPE: low-density polyethylene, and PS: polystyrene) at 1 mg/mL for pentane, acrolein, propanal, 2-methyl pentane, and hexanal measured at 3 days and 7 days of incubation. Data are shown in ppbv and as mean ± standard deviation. Bold values denote statistical significance at the $p < 0.05$ level.

VOC	Sample	Exposure time (d)		*p-value*
		3	7	
Pentane	CTRL	2.1 ± 0.4	1.8 ± 0.2	0.4
	HDPE_0W	2.2 ± 0.4	1.9 ± 0.4	0.6
	HDPE_4W	4.0 ± 1.1	4.4 ± 0.9	0.7
	LDPE_0W	2.8 ± 0.4	2.6 ± 0.6	0.4
	LDPE_4W	6.0 ± 1.7	6.3 ± 0.9	0.6
	PS_0W	1.6 ± 0.3	2.0 ± 0.4	**<0.05**
	PS_4W	16.6 ± 2.2	12.2 ± 1.1	**<0.05**
Acrolein	CTRL	2.3 ± 0.4	3.0 ± 0.2	0.07
	HDPE_0W	10.9 ± 2.1	9.5 ± 1.9	0.6
	HDPE_4W	39.7 ± 11.4	45.0 ± 8.8	0.7
	LDPE_0W	4.6 ± 0.7	6.0 ± 1.4	0.08
	LDPE_4W	6.1 ± 1.8	11.8 ± 1.7	**<0.0001**
	PS_0W	3.0 ± 0.2	3.0 ± 0.5	**<0.05**
	PS_4W	9.5 ± 1.9	46.4 ± 4.2	**<0.05**
Propanal	CTRL	47 ± 8	37 ± 5	0.3
	HDPE_0W	14 ± 3	12 ± 2	0.5
	HDPE_4W	105 ± 30	115 ± 23	0.8
	LDPE_0W	41 ± 6	61 ± 15	0.06
	LDPE_4W	136 ± 39	108 ± 16	0.2
	PS_0W	24 ± 4	15 ± 3	**<0.05**
	PS_4W	485 ± 65	314 ± 30	**<0.05**
2-methyl pentane	CTRL	3.4 ± 0.6	3.1 ± 0.3	0.7
	HDPE_0W	3.5 ± 0.7	3.4 ± 0.4	0.6
	HDPE_4W	49 ± 8	45 ± 4	0.6
	LDPE_0W	12 ± 2	19 ± 5	0.05
	LDPE_4W	67 ± 19	46 ± 6	0.1
	PS_0W	60 ± 9	46 ± 5	0.1
	PS_4W	71 ± 4	58 ± 9	0.1
Hexanal	CTRL	26 ± 5	20 ± 5	0.1
	HDPE_0W	33 ± 6	68 ± 6	**<0.05**
	HDPE_4W	920 ± 100	814 ± 160	0.4
	LDPE_0W	26 ± 3	67 ± 4	**<0.01**
	LDPE_4W	1080 ± 185	563 ± 83	**<0.05**
	PS_0W	35 ± 6	128 ± 23	**<0.05**
	PS_4W	930 ± 125	929 ± 85	0.9

comparatively. While these results may represent a breakthrough in understanding how MPs impact biological systems and show the advantages of including a characterisation of chemical by-products, it should be pointed out that the present study is only a first step towards a more comprehensive investigation that should explore the many specific features of such complex class of contaminants and their role in the physiological response. On the other hand, our results provide clear evidence of the presence of VOCs as a class of indicators of the multiple cell responses to the insult from MPs contamination, the variability of VOCs composition and cell response parameters, and the connections with exposure to MPs. However, further investigations are needed to explore whether the correlations between VOCs and inflammation-oxidative stress data can be confirmed and prove a cause-and-effect relation, allowing us to exploit them as biological markers for the activation of specific cellular pathways.

Finally, it will be important to understand which is the primary mediator of the biological effect in terms of the release of a degradation product, namely the physical contact between MPs and cells and/or the organic compounds leached out of MPs.

6. Conclusion

This study highlighted the effects of aged plastic particles on VSMCs through a general upregulation of oxidative stress and inflammation compounds. By means of an innovative analytical protocol that combines needle trap micro-extraction coupled with headspace analysis and biological assays, we succeeded in obtaining evidence and assessing the impact of aged MPs on VSMCs.

To the best of our knowledge, this is the first example in which the response and metabolic activity of human cells, here VSMCs, to the exposure to both virgin and artificially aged MPs of different polymer types, has been evaluated.

Our findings suggest that the effects of MPs on VSMCs may be characterized by low toxicity and slow damage, with inflammation and oxidative stress as the main underlying mechanisms. Understanding the toxicological mechanisms of aged plastic particles may potentially contribute to a better understanding of the pathogenesis of cardiovascular disease. In summary, the pathological status of VSMCs prompted by MP exposure has been proved via biological assays in addition to the exhaled chemical profile, and it is relevant in the realm of cardiovascular risk. Identification of biological markers, in addition to exhaled VOCs, could be helpful in the assessment of cardiovascular events driven by MP exposure. Even if more research and refinements are required, these novel molecular fingerprints (both biological indicators and VOCs) might be applied in MP health hazard estimation for their ease of use, applicability and reliability.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data Availability

Data will be made available on request.

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Author contributions statement

E.P., F.V., and T.L. conceived the project and designed experiments. E.P., D.B., I.G., E.C., A.Ce., A.Co., S.G. conducted the experiments. E.P., D.B., F.V., and T.L. performed data analysis. E.P., F.V., and T.L. wrote the manuscript. F.D.F., V.C., E.P., F.V., and T.L. carefully edited the manuscript. F.V. and T.L. funding acquisition.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.116695.

References

Akdogan, Z., Guven, B., 2019. Microplastics in the environment: a critical review of current understanding and identification of future research needs. Environ. Pollut. 254 https://doi.org/10.1016/J.ENVPOL.2019.113011.

Aranda-Rivera, A.K., Cruz-Gregorio, A., Lyzet Arancibia-Hernández, Y., Yaquelin Hernández-Cruz, E., Pedraza-Chaverri, J., 2022. RONS and Oxidative Stress: An Overview of Basic Concepts. Oxygen 2, 437–478. https://doi.org/10.3390/OXYGEN2040030.

Banerjee, A., Shelver, W.L., 2020. Micro- and nanoplastic induced cellular toxicity in mammals: a review, 142518–142518 Sci. Total Environ. 755. https://doi.org/10.1016/J.SCITOTENV.2020.142518.

Barshtein, G., Livshits, L., Shvartsman, L.D., Shlomai, N.O., Yedgar, S., Arbell, D., 2016. Polystyrene nanoparticles activate erythrocyte aggregation and adhesion to endothelial cells. Cell Biochem. Biophys. 74, 19–27. https://doi.org/10.1007/S12013-015-0705-6.

Biagini, D., Lomonaco, T., Ghimenti, S., Onor, M., Bellagambi, F.G., Salvo, P., Di Francesco, F., Fuoco, R., 2019. Using labelled internal standards to improve needle trap micro-extraction technique prior to gas chromatography/mass spectrometry. Talanta 200, 145–155. https://doi.org/10.1016/J.TALANTA.2019.03.046.

Bischoff, A.C., Oertel, P., Sukul, P., Rimmbach, C., David, R., Schubert, J., Miekisch, W., 2018. Smell of cells: Volatile profiling of stem- and non-stem cell proliferation. J. Breath. Res. 12, 026014 https://doi.org/10.1088/1752-7163/AAA111.

Braun, T., Ehrlich, L., Henrich, W., Koeppel, S., Lomako, I., Schwabl, P., Liebmann, B., 2021. Detection of Microplastic in Human Placenta and Meconium in a Clinical Setting. Pharmaceutics 13. https://doi.org/10.3390/PHARMACEUTICS13070921.

Calenic, B., Miricescu, D., Greabu, M., Kuznetsov, A.V., Troppmair, J., Ruzsanyi, V., Amann, A., 2015. Oxidative stress and volatile organic compounds: interplay in pulmonary, cardio-vascular, digestive tract systems and cancer. Open Chem. 13 https://doi.org/10.1515/chem-2015-0105.

Camps, J., García-Heredia, A., 2014. Introduction: oxidation and inflammation, a molecular link between non-communicable diseases. Adv. Exp. Med. Biol. 824, 1–4. https://doi.org/10.1007/978-3-319-07320-0_1.

Castelvetro, V., Corti, A., Biale, G., Ceccarini, A., Degano, I., La Nasa, J., Lomonaco, T., Manariti, A., Manco, E., Modugno, F., Vinciguerra, V., 2021. New methodologies for the detection, identification, and quantification of microplastics and their environmental degradation by-products. Environ. Sci. Pollut. Res. Int. 28, 46764. https://doi.org/10.1007/S11356-021-12466-Z.

Chen, Y.C., Chen, K.F., Andrew Lin, K.Y., Su, H.P., Wu, D.N., Lin, C.H., 2023. Evaluation of toxicity of polystyrene microplastics under realistic exposure levels in human vascular endothelial EA.hy926 cells. Chemosphere 313, 137582. https://doi.org/10.1016/J.CHEMOSPHERE.2022.137582.

DeJarnett, N., Conklin, D.J., Riggs, D.W., Myers, J.A., O'Toole, T.E., Hamzeh, I., Wagner, S., Chugh, A., Ramos, K.S., Srivastava, S., Higdon, D., Tollerud, D.J., DeFilippis, A., Becher, C., Wyatt, B., McCracken, J., Abplanalp, W., Rai, S.N., Ciszewski, T., Xie, Z., Yeager, R., Prabhu, S.D., Bhatnagar, A., 2014. Acrolein exposure is associated with increased cardiovascular disease risk. J. Am. Heart Assoc. 3 https://doi.org/10.1161/JAHA.114.000934.

Deng, Y., Zhang, Y., Lemos, B., Ren, H., 2017. Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. Sci. Rep. 7 https://doi.org/10.1038/SREP46687.

Dettmer, K., Engewald, W., 2002. Adsorbent materials commonly used in air analysis for adsorptive enrichment and thermal desorption of volatile organic compounds. Anal. Bioanal. Chem. 373, 490–500. https://doi.org/10.1007/S00216-002-1352-5.

Dong, C.Di, Chen, C.W., Chen, Y.C., Chen, H.H., Lee, J.S., Lin, C.H., 2020. Polystyrene microplastic particles: In vitro pulmonary toxicity assessment. J. Hazard. Mater. 385 https://doi.org/10.1016/J.JHAZMAT.2019.121575.

Du, H., Xie, Y., Wang, J., 2021. Microplastic degradation methods and corresponding degradation mechanism: Research status and future perspectives. J. Hazard. Mater. 418, 126377 https://doi.org/10.1016/J.JHAZMAT.2021.126377.

Dwivedi, A.M., Upadhyay, S., Johanson, G., Ernstgård, L., Palmberg, L., 2018. Inflammatory effects of acrolein, crotonaldehyde and hexanal vapors on human primary bronchial epithelial cells cultured at air-liquid interface. Toxicol. Vitr. 46, 219–228. https://doi.org/10.1016/J.TIV.2017.09.016.

Eliso, M.C., Billè, B., Cappello, T., Maisano, M., 2024. Polystyrene Micro- and Nanoplastics (PS MNPs): A Review of Recent Advances in the Use of -Omics in PS MNP Toxicity Studies on Aquatic Organisms, 9, 98 Fishes 2024 Vol. 9, 98. https://doi.org/10.3390/FISHES9030098.

Emenike, E.C., Okorie, C.J., Ojeyemi, T., Egbemhenghe, A., Iwuozor, K.O., Saliu, O.D., Okoro, H.K., Adeniyi, A.G., 2023. From oceans to dinner plates: The impact of microplastics on human health. Heliyon 9. https://doi.org/10.1016/J.HELIYON.2023.E20440.

Endale, H.T., Tesfaye, W., Mengstie, T.A., 2023. ROS induced lipid peroxidation and their role in ferroptosis. Front. Cell Dev. Biol. 11, 1226044. https://doi.org/10.3389/FCELL.2023.1226044/BIBTEX.

González-Acedo, A., García-Recio, E., Illescas-Montes, R., Ramos-Torrecillas, J., Melguizo-Rodríguez, L., Costela-Ruiz, V.J., 2021. Evidence from in vitro and in vivo studies on the potential health repercussions of micro- and nanoplastics. Chemosphere 280. https://doi.org/10.1016/J.CHEMOSPHERE.2021.130826.

Halfar, J., Čabanová, K., Vávra, K., Delongová, P., Motyka, O., Špaček, R., Kukutschová, J., Šimetka, O., Heviánková, S., 2023. Microplastics and additives in patients with preterm birth: The first evidence of their presence in both human amniotic fluid and placenta. Chemosphere 343. https://doi.org/10.1016/J.CHEMOSPHERE.2023.140301.

Hesler, M., Aengenheister, L., Ellinger, B., Drexel, R., Straskraba, S., Jost, C., Wagner, S., Meier, F., von Briesen, H., Büchel, C., Wick, P., Buerki-Thurnherr, T., Kohl, Y., 2019. Multi-endpoint toxicological assessment of polystyrene nano- and microparticles in different biological models in vitro. Toxicol Vitr. 61, 104610. https://doi.org/10.1016/J.TIV.2019.104610.

Hu, D., Yin, C., Luo, S., Habenicht, A.J.R., Mohanta, S.K., 2019. Vascular Smooth Muscle Cells Contribute to Atherosclerosis Immunity. Front. Immunol. 10, 1101. https://doi.org/10.3389/FIMMU.2019.01101.

Hwang, J., Choi, D., Han, S., Choi, J., Hong, J., 2019. An assessment of the toxicity of polypropylene microplastics in human derived cells. Sci. Total Environ. 684, 657–669. https://doi.org/10.1016/J.SCITOTENV.2019.05.071.

Jolliffe, I.T., 2002. Principal Component Analysis , 2002. Springer Series in Statistics. https://doi.org/10.1007/B98835.

Klemenz, A.C., Meyer, J., Ekat, K., Bartels, J., Traxler, S., Schubert, J.K., Kamp, G., Miekisch, W., Peters, K., 2019. Differences in the Emission of Volatile Organic Compounds (VOCs) between Non-Differentiating and Adipogenically Differentiating Mesenchymal Stromal/Stem Cells from Human Adipose Tissue. Cells 8. https://doi.org/10.3390/CELLS8070697.

Kü, A., Fischer, S., Bergmann, A., Oertel, P., Steffens, M., Trefz, P., Miekisch, W., Schubert, J.K., Reinhold, P., Köhler, H., 2016. Effects of biological and methodological factors on volatile organic compound patterns during cultural growth of Mycobacterium avium ssp. paratuberculosis. J. Breath. Res. 10 https://doi.org/10.1088/1752-7155/10/3/037103.

La Nasa, J., Lomonaco, T., Manco, E., Ceccarini, A., Fuoco, R., Corti, A., Modugno, F., Castelvetro, V., Degano, I., 2021. Plastic breeze: Volatile organic compounds (VOCs) emitted by degrading macro- and microplastics analyzed by selected ion flow-tube mass spectrometry. Chemosphere 270, 128612. https://doi.org/10.1016/J.CHEMOSPHERE.2020.128612.

Lehner, R., Wohlleben, W., Septiadi, D., Landsiedel, R., Petri-Fink, A., Rothen-Rutishauser, B., 2020. A novel 3D intestine barrier model to study the immune response upon exposure to microplastics. Arch. Toxicol. 94, 2463–2479. https://doi.org/10.1007/S00204-020-02750-1.

Li, B., Ding, Y., Cheng, X., Sheng, D., Xu, Z., Rong, Q., Wu, Y., Zhao, H., Ji, X., Zhang, Y., 2020. Polyethylene microplastics affect the distribution of gut microbiota and inflammation development in mice. Chemosphere 244, 125492. https://doi.org/10.1016/J.CHEMOSPHERE.2019.125492.

Little, L.D., Carolan, V.A., Allen, K.E., Cole, L.M., Haywood-Small, S.L., 2020. Headspace analysis of mesothelioma cell lines differentiates biphasic and epithelioid sub-types. J. Breath. Res 14, 046011. https://doi.org/10.1088/1752-7163/ABAAFF.

Lomonaco, T., Manco, E., Corti, A., La Nasa, J., Ghimenti, S., Biagini, D., Di Francesco, F., Modugno, F., Ceccarini, A., Fuoco, R., Castelvetro, V., 2020. Release of harmful volatile organic compounds (VOCs) from photo-degraded plastic debris: A neglected source of environmental pollution. J. Hazard. Mater. 394, 122596 https://doi.org/10.1016/J.JHAZMAT.2020.122596.

Lord, H.L., Zhan, W., Pawliszyn, J., 2010. Fundamentals and applications of needle trap devices: A critical review. Anal. Chim. Acta 677, 3–18. https://doi.org/10.1016/J.ACA.2010.06.020.

Marfella, R., Prattichizzo, F., Sardu, C., Fulgenzi, G., Graciotti, L., Spadoni, T., D'Onofrio, N., Scisciola, L., La Grotta, R., Frigé, C., Pellegrini, V., Municinò, M., Siniscalchi, M., Spinetti, F., Vigliotti, G., Vecchione, C., Carrizzo, A., Accarino, G., Squillante, A., Spaziano, G., Mirra, D., Esposito, R., Altieri, S., Falco, G., Fenti, A., Galoppo, S., Canzano, S., Sasso, F.C., Matacchione, G., Olivieri, F., Ferraraccio, F., Panarese, I., Paolisso, P., Barbato, E., Lubritto, C., Balestrieri, M.L., Mauro, C., Caballero, A.E., Rajagopalan, S., Ceriello, A., D'Agostino, B., Iovino, P., Paolisso, G., 2024. Microplastics and Nanoplastics in Atheromas and Cardiovascular Events. N. Engl. J. Med. 390, 900–910. https://doi.org/10.1056/NEJMOA2309822.

Mohammad, M.K., Avila, D., Zhang, J., Barve, S., Arteel, G., McClain, C., Joshi-Barve, S., 2012. Acrolein cytotoxicity in hepatocytes involves endoplasmic reticulum stress, mitochondrial dysfunction and oxidative stress. Toxicol. Appl. Pharmacol. 265, 73–82. https://doi.org/10.1016/J.TAAP.2012.09.021.

Molla, M.D., Akalu, Y., Geto, Z., Dagnew, B., Ayelign, B., Shibabaw, T., 2020. Role of Caspase-1 in the Pathogenesis of Inflammatory-Associated Chronic Noncommunicable Diseases. J. Inflamm. Res. 13, 749. https://doi.org/10.2147/JIR.S277457.

Muzio, G., Maggiora, M., Paiuzzi, E., Oraldi, M., Canuto, R.A., 2012. Aldehyde dehydrogenases and cell proliferation. Free Radic. Biol. Med. 52, 735–746. https://doi.org/10.1016/J.FREERADBIOMED.2011.11.033.

Ogale, N., Kamble, K., 2022. A review of the impacts on environment of microplastic beads Vs biodegradable beads used in cosmetics and personal care. Int. J. Pharm. Res. Appl. 7, 1421. https://doi.org/10.35629/7781-070214211425.

Ogbodo, J.O., Arazu, A.V., Iguh, T.C., Onwodi, N.J., Ezike, T.C., 2022. Volatile organic compounds: A proinflammatory activator in autoimmune diseases. Front. Immunol. 13, 928379 https://doi.org/10.3389/FIMMU.2022.928379/BIBTEX.

Oliveri, P., Forina, M., 2012. Data Analysis and Chemometrics. Chem. Anal. Food Tech. Appl. 25–57. https://doi.org/10.1016/B978-0-12-384862-8.00002-9.

Prata, J.C., Patrício Silva, A.L., da Costa, J.P., Mouneyrac, C., Walker, T.R., Duarte, A.C., Rocha-Santos, T., 2019. Solutions and Integrated Strategies for the Control and Mitigation of Plastic and Microplastic Pollution. Int. J. Environ. Res. Public Heal. 2019 16, 2411. https://doi.org/10.3390/IJERPH16132411.

Prietl, B., Meindl, C., Roblegg, E., Pieber, T.R., Lanzer, G., Fröhlich, E., 2014. Nano-sized and micro-sized polystyrene particles affect phagocyte function. Cell Biol. Toxicol. 30 (1) https://doi.org/10.1007/S10565-013-9265-Y.

Prozialeck, W.C., Edwards, J.R., Nebert, D.W., Woods, J.M., Barchowsky, A., Atchison, W.D., 2008. The vascular system as a target of metal toxicity. Toxicol. Sci. 102, 207–218. https://doi.org/10.1093/TOXSCI/KFM263.

Qiao, R., Sheng, C., Lu, Y., Zhang, Y., Ren, H., Lemos, B., 2019. Microplastics induce intestinal inflammation, oxidative stress, and disorders of metabolome and microbiome in zebrafish. Sci. Total Environ. 662, 246–253. https://doi.org/10.1016/J.SCITOTENV.2019.01.245.

Ragusa, A., Svelato, A., Santacroce, C., Catalano, P., Notarstefano, V., Carnevali, O., Papa, F., Rongioletti, M.C.A., Baiocco, F., Draghi, S., D'Amore, E., Rinaldo, D., Matta, M., Giorgini, E., 2021. Plasticenta: First evidence of microplastics in human placenta. Environ. Int. 146 https://doi.org/10.1016/J.ENVINT.2020.106274.

Rastogi, S., Rizwani, W., Joshi, B., Kunigal, S., Chellappan, S.P., 2012. TNF-α response of vascular endothelial and vascular smooth muscle cells involve differential utilization of ASK1 kinase and p73. Cell Death Differ. 19, 274–283. https://doi.org/10.1038/CDD.2011.93.

Schirinzi, G.F., Pérez-Pomeda, I., Sanchís, J., Rossini, C., Farré, M., Barceló, D., 2017. Cytotoxic effects of commonly used nanomaterials and microplastics on cerebral and epithelial human cells. Environ. Res. 159, 579–587. https://doi.org/10.1016/J.ENVRES.2017.08.043.

Sharma, A., Kumar, R., Varadwaj, P., 2023. Smelling the Disease: Diagnostic Potential of Breath Analysis. Mol. Diagn. Ther. 27, 321–347. https://doi.org/10.1007/S40291-023-00640-7.

Stock, V., Böhmert, L., Dönmez, M.H., Lampen, A., Sieg, H., 2020. An inverse cell culture model for floating plastic particles. Anal. Biochem. 591, 113545 https://doi.org/10.1016/J.AB.2019.113545.

Traxler, S., Barkowsky, G., Saß, R., Klemenz, A.C., Patenge, N., Kreikemeyer, B., Schubert, J.K., Miekisch, W., 2019. Volatile scents of influenza A and S. pyogenes (co-)infected cells. Sci. Rep. 2019 91 9 (1), 12. https://doi.org/10.1038/s41598-019-55334-0.

Trefz, P., Koehler, H., Klepik, K., Moebius, P., Reinhold, P., Schubert, J.K., Miekisch, W., 2013. Volatile Emissions from Mycobacterium avium subsp. paratuberculosis Mirror Bacterial Growth and Enable Distinction of Different Strains. PLoS One 8, e76868. https://doi.org/10.1371/journal.pone.0076868.

Vaduganathan, M., Mensah, G.A., Turco, J.V., Fuster, V., Roth, G.A., 2022. The Global Burden of Cardiovascular Diseases and Risk: A Compass for Future Health. J. Am. Coll. Cardiol. 80, 2361–2371. https://doi.org/10.1016/J.JACC.2022.11.005.

Wang, Y.L., Lee, Y.H., Hsu, Y.H., Chiu, I.J., Huang, C.C.Y., Huang, C.C., Chia, Z.C., Lee, C. P., Lin, Y.F., Chiu, H.W., 2021. The Kidney-Related Effects of Polystyrene Microplastics on Human Kidney Proximal Tubular Epithelial Cells HK-2 and Male C57BL/6 Mice. Environ. Health Perspect. 129 https://doi.org/10.1289/EHP7612.

Wright, S.L., Kelly, F.J., 2017. Plastic and Human Health: A Micro Issue? Environ. Sci. Technol. 51, 6634–6647. https://doi.org/10.1021/ACS.EST.7B00423/ASSET/IMAGES/LARGE/ES-2017-004235_0003.JPEG.

Wu, B., Wu, X., Liu, S., Wang, Z., Chen, L., 2019. Size-dependent effects of polystyrene microplastics on cytotoxicity and efflux pump inhibition in human Caco-2 cells. Chemosphere 221, 333–341. https://doi.org/10.1016/J.CHEMOSPHERE.2019.01.056.

Wu, D., Feng, Y., Wang, R., Jiang, J., Guan, Q., Yang, X., Wei, H., Xia, Y., Luo, Y., 2023. Pigment microparticles and microplastics found in human thrombi based on Raman spectral evidence. J. Adv. Res. 49, 141–150. https://doi.org/10.1016/J.JARE.2022.09.004.

Wu, X., Pan, J., Li, M., Li, Y., Bartlam, M., Wang, Y., 2019. Selective enrichment of bacterial pathogens by microplastic biofilm. Water Res 165. https://doi.org/10.1016/J.WATRES.2019.114979.

Yang, B.C., Yang, Z.H., Pan, X.J., Xiao, F.J., Liu, X.Y., Zhu, M.X., Xie, J.P., 2013. Crotonaldehyde-exposed macrophages induce IL-8 release from airway epithelial cells through NF-κB and AP-1 pathways. Toxicol. Lett. 219, 26–34. https://doi.org/10.1016/J.TOXLET.2013.02.018.

Yang, Y.F., Chen, C.Y., Lu, T.H., Liao, C.M., 2019. Toxicity-based toxicokinetic/toxicodynamic assessment for bioaccumulation of polystyrene microplastics in mice. J. Hazard. Mater. 366, 703–713. https://doi.org/10.1016/J.JHAZMAT.2018.12.048.

Yin, L., Chen, B., Xia, B., Shi, X., Qu, K., 2018. Polystyrene microplastics alter the behavior, energy reserve and nutritional composition of marine jacopever (Sebastes schlegelii). J. Hazard. Mater. 360, 97–105. https://doi.org/10.1016/J.JHAZMAT.2018.07.110.

Yu, Y., Chen, H., Hua, X., Dang, Y., Han, Y., Yu, Z., Chen, X., Ding, P., Li, H., 2020. Polystyrene microplastics (PS-MPs) toxicity induced oxidative stress and intestinal injury in nematode Caenorhabditis elegans. Sci. Total Environ. 726 https://doi.org/10.1016/J.SCITOTENV.2020.138679.

Zhu, X., Wang, C., Duan, X., Liang, B., Genbo Xu, E., Huang, Z., 2023. Micro- and nanoplastics: A new cardiovascular risk factor? Environ. Int. 171, 107662 https://doi.org/10.1016/J.ENVINT.2022.107662.

Zitouni, N., Cappello, T., Missawi, O., Boughattas, I., De Marco, G., Belbekhouche, S., Mokni, M., Alphonse, V., Guerbej, H., Bousserrhine, N., Banni, M., 2022. Metabolomic disorders unveil hepatotoxicity of environmental microplastics in wild fish Serranus scriba (Linnaeus 1758). Sci. Total Environ. 838, 155872 https://doi.org/10.1016/J.SCITOTENV.2022.155872.