Detection of microplastics in human lung tissue using µFTIR spectroscopy

Abstract

Airborne microplastics (MPs) have been sampled globally, and their concentration is known to increase in areas of high human population and activity, especially indoors. Respiratory symptoms and disease following exposure to occupational levels of MPs within industry settings have also been reported. It remains to be seen whether MPs from the environment can be inhaled, deposited and accumulated within the human lungs. This study analysed digested human lung tissue samples (n=13) using µFTIR spectroscopy (size limitation of 3 µm) to detect and characterise any MPs present. In total, 39 MPs were identified within 11 of the 13 lung tissue samples with an average of 1.42±1.50 MP/g of tissue (expressed as 0.69±0.84 MP/g after background subtraction adjustments). The MP levels within tissue samples were significantly higher than those identified within combined procedural/laboratory blanks (n=9 MPs, with a mean±SD of 0.53±1.07, p=0.001). Of the MPs detected, 12 polymer types were identified with polypropylene, PP (23%), polyethylene terephthalate, PET (18%) and resin (15%) the most abundant. MPs (unadjusted) were identified within all regions of the lung categorised as upper (0.80±0.96 MP/g), middle/lingular (0.41±0.37 MP/g), and with significantly higher levels detected in the lower (3.12±1.30 MP/g) region compared with the upper (p=0.026) and mid (p=0.038) lung regions. After subtracting blanks, these levels became 0.23±0.28, 0.33±0.37 and 1.65±0.88 MP/g respectively. The study demonstrates the highest level of contamination control and reports unadjusted values alongside different contamination adjustment techniques. These results support inhalation as a route of exposure for environmental MPs, and this characterisation of types and levels can now inform realistic conditions for laboratory exposure experiments, with the aim of determining health impacts.

Abbreviations

LOD – limit of detection

LOQ – limit of quantitation

μFTIR – micro Fourier Transform Infrared

MCT – mercury cadmium telluride

MP – microplastics between 1 μm and 5 mm

NP – nanoplastics

PAN - polyacrylonitrile

PE - polyethylene

PES - polyester

PET - polyethylene terephthalate

PMMA - polymethylmethacrylate

PP – polypropylene

PS - polystyrene,

PTFE - polytetrafluoroethylene

PUR – polyurethane

PVA – polyvinyl alcohol

ROS – reactive oxygen species

SEBS - styrene-ethylene-butylene co-polymer

TPE - thermoplastic elastomer

1. Introduction

Microplastics (MPs), defined herein as plastic particles between 1 μm and 5 mm [1], are present in all environmental compartments; from marine and freshwater bodies [2], to soil [3], food, drinking water [4,5], and air [6–9]. For the latter, suspended MP particles have been isolated from many atmospheric locations, including urbanised city centres [8,10,11], indoor households [7,9,12,13], and remote outdoor regions [6]. Previous work highlights that citizens are exposed to higher concentrations of MP within their homes [9], compared to outdoors, and this results in ubiquitous and unavoidable human exposure [14]. Consequentially, there is an increasing concern regarding the hazards associated with MP ingestion, dermal contact, and inhalation [14].

Synthetic fibres have previously been observed within human lung tissue samples [15], yet limited studies confirm the presence of MPs within the lungs alongside chemical analysis tools, such as μRaman and μFTIR spectroscopy [16]. Reliance upon observational criteria alone to distinguish between MP and non-MPs, can lead to over and under-estimated MP counts, and a lack of information relating to polymer or additive type [17,18]. The plausibility of MP inhalation has been highlighted [19,20] and MPs with a width as small as 5 μm have been reported within air samples [10,21]. Upon environmental release, plastics are exposed to oxidation, mechanical stress and biological action, resulting in embrittlement and fragmentation, forming MPs, and eventually nanoplastics (NPs)(<1μm), as well as release into the environment in their primary form [18].

Historical studies report respiratory symptoms and disease at an occupational level of exposure in synthetic textile, flock, and vinyl chloride workers [19], and as such, support inhalation as an exposure route for MPs. However, it remains unclear whether MPs can enter and remain in the lungs of the general population due to environmental exposure, rather than the chronic levels seen within industry settings. MPs are designed to be robust materials, unlikely to break down within the

lungs [22], potentially leading to accumulation over time depending on aerodynamic diameter and respiratory defences [19].

The mounting concern surrounding airborne MPs stems from the unknown polymer types, levels of exposure, and consequences of their inhalation. MP characteristics such as size, shape, vectored absorbed pollutants and pathogens, as well as plastic monomer or additive leaching, have been highlighted as potential promoters of cytotoxicity [20]. MPs are consistently identified within air samples, their concentration is highest indoors [7,12,13] and within highly populated areas [8], they are readily suspended at times of high human activity [13] and are often small and fibrous [11]. Together, these concerns highlight the necessity for accurate tissue analysis to understand the potential for these synthetic polymers to penetrate the human respiratory system and cause harm.

This study aims to identify any MP particles present in digested human lung tissue samples, while also accounting for procedural and laboratory blank contamination. Any particles isolated from lung tissue have been chemically characterised using μFTIR spectroscopy (with a 3 μm lower size limit of detection).

2. Material and Methods

2.1 Human tissue acquisition

Excess human lung tissue was collected from thoracic surgical procedures at Castle Hill Hospital, Hull University Teaching Hospitals NHS Trust, following NHS Research Ethics Committee and Health Research Authority approval (REC reference 12/SC/0474). Samples of peripheral human lung tissue were collected from upper, middle (left lingula) or lower lobe specimens following surgical resection for cancer or lung volume reduction surgery. Descriptions of the tissue origin were provided by the surgical team. Care was taken to avoid the tumour margins. Details of the donors smoking status, occupation and area of residence were unavailable for the researchers under the terms

of the ethical approval obtained. Tissue samples were placed into empty glass containers with foil lids and immediately frozen (-80°C) until bulk analysis (two batches) was conducted. Lung tissue was obtained from 11 patients (numbered 1.1 to 11.1), with patients 1 and 2 providing two samples (numbered 1.2 and 2.2) from different lung positions (n=13, total tissue mass=55.41 g), resulting in a mean mass of 4.26±3.87 g (range 0.79-13.33 g). Patients mean age was 63±13 years (range 32-77), 5 females and 6 males (Table 1).

2.2 Lung tissue digestion and filtration

Thawed samples were exposed to a hydrogen peroxide (100 mL of 30% H_2O_2) bath and rinsed alongside 'procedural blanks' (n=4) (Supplementary Fig. S1). Each tissue sample was transferred to a clean glass conical flask with a foil covering, and 100 mL of 30% H_2O_2 added. The total mass of each individual tissue sample digested is detailed in Table 1. Flasks were placed in a shaking incubator at 55°C for approximately 11 days, 65 rpm, or until there was no visible tissue. After 5 days within the incubator, an additional 100 mL of 30% H_2O_2 was added. The digest, adapted from previous studies investigating MPs within different environmental and tissue samples [23], ensures removal of organic particles whilst maintaining MP integrity [6,23]. Samples were then filtered onto aluminium oxide filters (0.02 µm Anodisc, Watford, U.K.) using a glass vacuum filtration system. These were stored in clean glass petri dishes, in the dark, before chemical composition analysis alongside laboratory blanks (n=13) (Supplementary Fig. S1).

2.3 Chemical characterisation of particles using µFTIR analysis

Each tissue sample Anodisc filter was placed directly onto the µFTIR spectroscopy platform, and the length (largest side) and width (second largest side) recorded using the aperture height, width and angle size selection tool, available within the ThermoScientific Omnic Picta Nicolet iN10

microscopy software. Particles were then assigned to a shape category (fibre, film, fragment, foam, or sphere [24]), whereby fibrous particles were characterised as having a length to width ratio >3 [12].

μFTIR spectroscopy analysis was conducted in liquid nitrogen cooled transmission mode (Nicolet iN10, ThermoFisher, Waltham MA, U.S.A), without the aid of further accessories or crystals. The cooled mercury cadmium telluride (MCT) detector allowed for the analysis of particles accurately down to 3 μm in size. No observational criteria [18] was applied to select specific particles for μFTIR analysis, to prevent bias. Using the aperture size selection tool, all particles upon the sample filter >3 μm were included in the analysis process. For this study, the whole filter, containing the total digested tissue sample, was analysed.

A background reference spectrum was first recorded, using identical parameters to the particles undergoing analysis. A blank area of the Anodisc filter was chosen as the site for background collection before immediate analysis of the sample particles. μFTIR parameters were; spectral range of 4000-1250 cm^{-1}, high spectral resolution 4 cm^{-1}, scan number of 64. No smoothing, baseline correction or data transformation was attempted. Resulting sample spectra were compared to a combination of polymer libraries (Omnic Picta, Omnic Polymer Libraries), available with the Omnic Picta software, and full spectral ranges were used with a match threshold of ≥70%. If particles were below the ≥70% match index threshold, three attempts were made to collect a successful match before moving on to the next particle undergoing analysis. Particles below ≥70% match, and particles not classified as a plastic were recorded but not included in the results presented [25].

During μFTIR analysis, one ‘laboratory blank’ Anodisc filter was opened alongside every sample filter (Supplementary Fig. S1). A total of 13 lung tissue samples were analysed, plus 4 ‘procedural blanks’, and 13 ‘laboratory blanks’. The total number of particles (MPs and others) identified was 296, whereby 225 (76%) of these were above the 70% hit quality index threshold. Only the MPs data is shown in the results. Identified PET and PES MP particles were reported

142 separately within this study, using a high match (>70 %) on a polymer database search to confirm
143 their identities.

144

145 *2.4 Quality assurance and control measures to reduce and quantify background MP contamination*

146 Strict control measures were adhered to, in order to quantify and characterise the nature of any
147 unavoidable background contamination. Due to the ubiquitous nature of MPs in the air, contamination
148 upon the surface of lung tissue samples could be possible during the surgical procedure, where lung
149 tissue was removed from live human subjects. While it was not possible to fully control the surgical
150 environment, each tissue sample was dropped into a 100 mL 30% H_2O_2 bath, re-sealed with foil and
151 agitated for 2 minutes. In parallel, 'procedural blanks' (n=4) were initiated. The tissue sample was
152 removed, and the outer surface rinsed thoroughly with 100 mL 30% H_2O_2 to remove any surface
153 contamination, employing a method similar to extracting microplastics from whole biota [26].
154 Analysis of solely the interior portion of the tissue was considered [15] but was not applied with the
155 aim of maintaining a larger tissue mass. Tissue samples were digested in two batches, with two
156 procedural blanks, which mimicked the entire tissue processing steps but lacked the lung tissue
157 sample, alongside each batch (Supplementary Fig. S1). Reagents were filtered and prepared in bulk
158 for each batch. When conducting μFTIR analyses, a 'laboratory blank' filter (n=13), placed in a glass
159 sealed petri dish, was opened for the same duration as that for the tissue sample.

160 MPs found within 'procedural blanks' represent contamination from the laboratory reagents,
161 equipment or fallout from the air during the transfer of samples. For each batch, the average
162 procedural contamination was calculated and assumed to be present within each of the tissue samples.
163 MPs within 'laboratory blanks' represent contamination from atmospheric fallout within the μFTIR
164 laboratory room during particle characterisation. Procedural blank and laboratory blank results were
165 combined to account for contamination at every step. No standardised protocols are currently adopted
166 within the MPs research field to account for background contamination, so multiple contamination

adjustments were applied in this study for comparison. These comprised two approaches: subtraction, routinely used in the MP research field, and a limit of detection (LOD) and limit of quantification (LOQ) technique [27] (Supplementary methods 1). Presenting raw data, subtraction, and LOD/LOQ adjusted results allows a comparison for each technique.

All H_2O_2 and MilliQ water used were triple filtered using an all-glass vacuum filtration kit and 47 mm glass fibre grade 6 filters (GE Healthcare Life Sciences, Marlborough MA, U.S.A). All glassware underwent thorough manual cleaning, before a dishwasher cycle using distilled water and then a manual three rinse wash with triple filtered MilliQ water. All equipment and reagents were always covered with foil lids and a small opening made when pouring. Additionally, when filtering digested samples, glassware and the sides of the filtration kit were rinsed three times with triple filtered MilliQ water to avoid sample particle loss. All work was conducted in a thoroughly cleaned fume cupboard with power 'off' and shield down to minimise unfiltered air flow [28] and particle suspension [29]. Each tissue sample was processed individually to prevent cross contamination. Plastic equipment was avoided, glass petri dishes, a cotton laboratory coat, and a new set of nitrile gloves for each sample processing step. Tissue preparation and particle analysis was conducted at times of low activity, no room ventilation and μFTIR conducted in a single person room with no windows. Finally, work was conducted by a single researcher for standardisation. To ensure no particles were contaminating the Anodisc filters from the manufacturing process of the discs used, three random filters were chosen and observed under the μFTIR, in which no particles were present.

2.5 Statistical analysis

Tests for homogeneity and significance were performed on unadjusted MP values using SPSS. All data were determined not normally distributed with a Shapiro-Wilk test and either a Kruskal-Wallis or Mann-Whitney U test applied.

3. Results

3.1 MP abundance levels detected in human lung tissue samples

A total of 39 MPs were identified within 11 of the 13 human lung tissue samples. An overall unadjusted mean of 3.00±2.55 MPs per sample (range 0-8 MPs) were identified within human lung tissue samples, significantly higher levels (p=0.001) compared with 0.53±1.07 MP per sample detected in the combined blanks. When considering the mass of the tissue sample, without accounting for background contamination, a mean of 1.42±1.50 MP/g was detected (Table 1). After subtracting background contamination, this value becomes 0.69±0.84 MP/g (Table 1). An unadjusted mean of 2.09±1.54 MP/g of tissue was identified in male (n=6) and 0.36±0.50 MP/g of tissue in female (n=5) samples (adjusted to 0.91±0.95 MP/g and 0.33±0.52 MP/g respectively after subtracting background contamination). All male samples contained at least one MP particle, whilst two of the five female samples did not. The data was not normally distributed *(p*=0.013), and a Mann-Whitney U test revealed tissue samples from male patients had significantly higher levels of MP/g compared to females (*p*=0.019). A detailed description of the characterisation of background MP contamination (procedural and laboratory blanks) can be found in the supplemental information (Table S1).

3.2 MP particle characterisation from human lung tissue samples

A total of 12 polymer types were identified in the tissue samples, as detailed in Fig 1 A. PP (9, 23%) and PET (7, 18%) were the most abundant (Fig 1A). All MPs identified within tissue samples were fibre (19, 49%), fragment (17, 43%), or film (3, 8%), (Fig 1B, Fig 2). MP particles identified within the tissue samples had a mean particle length of 223.10±436.16 μm (range 12-2475 μm), and a mean particle width of 22.21±20.32 μm (range 4-88 μm) (Fig 3A).

3.3 Characterisation of background MP contamination (procedural and laboratory blanks)

Considering all the blank samples, the mean background MP contamination rate detected was 0.53±1.07 MP per blank. Particles identified within 'procedural blanks' had a mean MP contamination rate of 2.00±2.83 MP per sample (range 0-4), for batch 1, whereby four MPs were identified on one filter: PE, PE/PP, PS, and a resin particle. No MPs were detected on the second filter for batch 1 (Table S1). No particles were identified within 'procedural blanks' from batch 2 of tissue samples on either of the two procedural blank filters (Table S1). Particles detected from 'laboratory blanks' (n=13) had an overall mean MP contamination rate of 0.38±0.65 MP per sample (range 0-2). This comprised one PET, PP, PS, PTFE and PVA particle from the 13 laboratory control filters (Table S1). The average length of MPs detected within the combined blank samples was 105.22±92.82 μm (range 23-315 μm), and an average width of 34.44 ± 22.61 μm (range 15-73 μm). The shapes of MPs identified in the combined blank samples were either fragment (6, 67%), fibre (2, 22%), or film (1, 11%).

In addition to MP particles, non-MP 'natural polymer' particles were detected on the sample filters. Combining non-MP procedural and laboratory blank results 9.04±4.84 non-MP particles per sample were detected, comprised of cellulose and zein.

3.4 Background MP contamination adjustments

Using adjustments, to account for the combined procedural and blank contamination levels detected, decreases the level of MPs identified within tissue samples depending on the approach used (Table 1). After blank subtraction adjustments, the total MPs identified within tissue samples have a mean of 0.69±0.84 MP/g of tissue. Subtraction adjusted MP levels in human lung tissues were statistically significant compared to blank data (Mann-Whitney U test, p=0.043). Only one lung tissue sample (sample 1.1) fit the criteria for using a LOD and LOQ calculation, showing 1.94 MP/g, above the quantification threshold. The polymer type detected above this threshold was PP. MPs above the

LOD, that can be detected within lung tissue samples, but not quantified, were PE, PET, PP, PTFE and resin.

3.5 MP distribution within human samples by lung region

MPs were identified within all regions of the lung (Fig 4 and Table S2). An unadjusted mean of 0.80±0.96 MP/g was identified within the upper region (adjusted to 0.23±0.28 MP/g after background subtraction), 0.41±0.37 MP/g within the middle/lingular region (adjusted to 0.33±0.37 MP/g) and 3.12±1.30 MP/g within the lower region (adjusted to 1.65±0.88 MP/g). Data was not normally distributed (p=0.013) and a Kruskal-Wallis test showed that the number of MPs in the lower region were significantly higher than the middle/lingular (p=0.038) and the upper region (p=0.026). Within the upper region (n=6, total mass=33.66 g), 11 MPs were identified; PE (2, 18%), PET (2, 18%), PP (2, 18%), PES (1, 9%), PS (1, 9%), resin (1, 9%), SEBS (1, 9%), TPE (1, 9%). Within the middle/lingular region (n=3, total tissue mass=12.19 g), 7 MPs were identified; PET (2, 29%), resin (2, 29%), PE (1, 14%), PMMA (1, 14%), PUR (1, 14%). Within the lower region (n=4, total tissue mass=9.56 g), 21 MPs were identified; PP (7, 33%), PTFE (4, 19%), PET (3, 14%), Resin (3, 14%), PS (2, 10%), PAN (1, 5%), PE (1, 5%) (Fig 4).

3.6 MP distribution within human lung tissue by individual patient

MPs were identified in 9 of the 11 patient lung samples. Multiple samples were taken from patient 1; 8 MPs in sample 1.1 and 2 MPs in sample 1.2 (Fig 5A). PP particles were identified within both samples (Fig 5B). Multiple samples were also taken from patient 2; 3 MPs in sample 2.1 and 3 MPs in sample 2.2. PTFE particles were identified within both samples, whilst multiple polymers were only identified within one patient sample (Fig 5B).

4. Discussion

This report provides compelling evidence of MPs within human lung tissue samples, using a robust, best practice, background contamination regime combined with μFTIR chemical composition analysis to verify the particles present. The study also highlights the importance of including and evaluating contamination adjustments within MP research, whilst providing high levels of quality assurance and control.

In total, 39 MPs were identified within 11 of the 13 lung tissue samples, with an unadjusted average of 1.42±1.50 MP/g of tissue. By subtracting any MPs detected in the corresponding blanks, an adjusted average of 0.69±0.84 MP/g tissue sample is reported. The MP levels within tissue samples were significantly higher than those identified within combined procedural/laboratory blanks. Of the MPs detected, 12 polymer types were identified with PP (23%), PET (18%), resin (15%), and PE (10%) the most abundant. It should be noted that the FTIR spectra for PET and PES (polyester) are similar and can be difficult to distinguish [30,31], however a high match of 70% was accepted to distinguish between the MP types within this study.

MPs were identified within all regions of the lung categorised as upper (0.80±0.96 MP/g), middle/lingular (0.41±0.37 MP/g), and lower (3.12±1.30 MP/g) region. However, when a LOD and LOQ approach was applied, only one tissue sample fit the criteria, with only PP detected above the threshold levels at 1.94 MP/g (Table 1). It could be that most MPs identified were contamination, however the LOD LOQ could also be 'masking' legitimately identified MPs. The LOD LOQ adjustment approach dramatically reduced the level of quantifiable MPs identified within lung tissue samples. This quality control measure has the benefit of providing a threshold above that of a simple subtraction, allowing MPs to be reliably detected and quantified [26]. Although it is an emerging technique within the MP field, it has the potential to account for polymer type as well as quantity and is commonly applied within analytical chemistry. However, samples containing low numbers of MPs, such as the human lung tissue samples reported here, commonly only have one MP particle per

288 polymer type identified in a sample. It has been reported that when dealing with such low MP
289 quantities within samples, the LOD LOQ technique will have more significant effects and lead to a
290 "reduced capacity to report any MPs above the LOD or LOQ" [27]. We therefore report our results
291 in three ways; unadjusted, subtraction adjusted and LOD LOQ adjusted, but highlight the importance
292 of the LOD LOQ technique for future studies in which MP abundance is not as low.

293 MPs have, to date, been detected in human samples from histological lung cancer samples
294 [15] and cadavers [16] as well as from human placenta [32]. Our findings are consistent with an early
295 study by Pauly et al (1998) using microscopy under polarised light to identify fibres (though without
296 chemical characterisation validation or rigorous contamination control measures), reporting presence
297 of fibres in 83% of nonneoplastic lung specimens (n=67/81) and in 97% of malignant lung specimens
298 (n=32/33)[15]. This study also reported that the fibres were distributed throughout all regions of the
299 lung and were not confined to the large air spaces [15]. While no formal size range is given in this
300 early study, they reported heterogeneity with respect to fibre length, width, surface morphology and
301 colour, with >250 μm length and ~50 μm width [15]. Our findings are also in line with a recent
302 publication by Amato-Lourenco *et al* who also found PP to be amongst the most abundant plastics
303 identified [16]. In contrast to our study, Amato-Lourenco *et al* showed that non-fibrous particles
304 were the most abundant type of MP with sizes smaller than those seen in our study. This could partly
305 by due to differing exposures to MP, our best practice approach used to eliminate background
306 contamination, or the methods used to detect and characterise samples, Raman vs. μFTIR. Although
307 Raman spectroscopy has the advantage of a lower method detection limit (~1μm), which might
308 explain the abundance of smaller particles identified in Amato-Lourenco's study [16], it can be
309 heavily influenced by fluorescence interference and does not detect the same polar peaks that μFTIR
310 spectroscopy can. Additionally, Raman spectroscopy can UV degrade the particles being analysed,
311 which could hinder potential future investigations. Thus, although both spectroscopic techniques
312 complement each other, μFTIR has some advantages that benefit MP research [33].

Interestingly, tissue from male donors contained significantly higher levels of unadjusted MP (2.09±1.54 MP/g) compared to females (0.36±0.50 MP/g), with all samples from males containing MPs but two out of five samples from females showing no MPs. We hypothesise that this is due female airways being significantly smaller than the airways of males [34], although the relatively small sample size used herein dictates that more analyses be conducted to explore such differences further.

According to Donaldson et al (1993), only particles with a physical diameter smaller than 3 μm can enter the alveolar region of the lung [35]. The alveolar duct is reported in the literature as being ~540 μm diameter and 1410 μm long [36]. Particles of a size ranging from 12-2,475 μm for length and 4-88 μm for width were detected within lung samples in this study, in theory, too large to be present, yet present nonetheless.

While the fate of particles entering the lung, and their resulting biological effects in terms of inflammation responses, are well established for ultrafine particulates in the NP or PM_{10} size range [37,38], the corresponding information is currently unavailable for the MP size range of particles observed here, highlighting a serious gap in the knowledge. There are limited recent studies giving evidence of particle sizes and deposition in the lungs. It could be that there may be a pre-conceived assumption about the particle sizes which are inhalable and able to make it into the lower airway, but in this study, and others [15, 16] particles bigger than these are being reported, and therefore, it may be time to revisit these numbers and investigate what sizes can be inhaled. Interestingly, even after LOD and LOQ were applied, the PP identified in sample 1.1 were all above the size limit which is generally thought of as inhalable.

12 MPs ≤10 μm were identified within 7 of the 13 lung tissue samples, consisting of PET (3), resin (3), PE (2), PP (2), PTFE (1) and PAN (1) (Table 1). The smallest particle identified was 14 μm in length and 4 μm width (Fig. 2C), and identified as an ‘alkyd resin’, a synthetic thermoplastic used in protective coatings and paints [39]. No MPs ≤10 μm were detected within blanks, surprising since

the prevalence of MPs in the environment is known to increase with decreasing particle size [6–8], suggesting that the quality assurance measures undertaken eliminated these smaller particles from blanks. As these small MPs were consistently absent from blanks (Fig. 3B), it highlights the likelihood of the smaller MPs being present within lung tissue rather than from background contamination sources.

The ubiquity of MPs within the environment, results in background contamination in any study, even after strict quality control measures are applied. Blanks, or controls, are run alongside sample analysis to document the levels and types of MPs contaminating samples, either by mimicking the sample processing steps ('procedural blank'), or by opening a clean filter during sample analysis ('laboratory blank'). Rarely are procedural and laboratory blanks both applied [26]. It was hypothesised in the design of this study that if MPs were present within lung tissue samples, they would be present at low levels, especially considering the detection limit of chemical verification. Thus, the importance of combining multiple procedural and laboratory blanks, is highlighted. In this study the MP characteristics identified within blanks were distinct from those identified within lung tissue samples; the main polymer abundance, size range and shape varied (Fig 3A, 3B). Human lung tissue samples were typically comprised of PP, PET and resin, with lengths ranging from 12-2475 μm and widths from 4-88 μm, and fibres being more prevalent than fragments. In contrast, MPs detected in the blanks were less abundant and comprised different particle characteristics. MPs were sized 23-315 μm and 15-73 μm for length and width, and fragments were more prevalent than fibres.

Within the MP literature, a standardised contamination adjustment technique has not been established. Therefore, this study opted to report concentrations in three commonly used ways; detailing blank results but making no adjustments [13,40], subtraction adjustments [6,41] and LOD LOQ adjustments [9,27]. Using no contamination adjustments, 1.42±1.50 MP/g of lung tissue was observed. While this method is common practice, it likely includes any contamination within the samples. The subtraction adjustment decreases the lung tissue MP final mean value to 0.69±0.84 MP/g and accounts for any potential background contamination but is not specific in terms of taking

into account particle characteristics. The LOD LOQ adjustment approach dramatically reduces the levels of MPs identified within the study to 0.15±0.54 MP/g using a polymer specific approach, but could be seen to 'mask' low levels of MPs. Ultimately this study highlights the need for data adjustments to account for background contamination, but alongside an evaluation into which adjustment is the best approach. Irrespective of the adjustments, low levels of MPs are present within lung tissue samples, providing evidence to support MP inhalation as a route of exposure to humans.

Airborne MPs are globally ubiquitous and especially prevalent indoors where humans spend many hours a day, such as the home [7,9,12,13] and the office [7,13]. Humans are thus continuously exposed to atmospheric MPs, with inhalation estimates ranging from 6-272 MP/day [12,19,42]. It is the smallest and least dense MP and NP particles that are the most cause for concern regarding respiratory health, as these MPs are most likely to deposit within the lungs based on aerodynamic diameter [19]. In contrast to NPs, MP particles in the full micro-size range (10 μm-5 mm) have yet to be considered in terms of health implications and potential impacts, perhaps not having been a priority compared with the smaller, ultrafine particles. The results herein indicate that the larger micro-size range are detected within human lung samples, suggesting that these have been overlooked (as being considered too large to enter lungs). MPs, like all macroplastics, are designed to be resilient, with the addition of dyes, and additives that dictate their properties [2]. It had previously been suggested that inhaled MPs are likely to bio-persist and possibly accumulate within a lung environment [20], showing resilience to degradation by synthetic extracellular lung fluid after 180 days [22]. After deposition within the lung, mechanisms of toxicity are unknown but particle properties such as small size, density, concentration, shape, monomer type, chemical leachates and environmental adsorbents (e.g. bacteria, heavy metals and polyaromatic hydrocarbons) have all been suggested as potential contributors to cytotoxicity [19,20]. Inflammation [43], ROS and oxidative stress [44], physical damage from particle shape, frustrated phagocytosis [35], are currently suggested cellular responses to MP exposure.

In summary, this study is the first to report MPs within human lung tissue samples, using μFTIR spectroscopy. The abundance of MPs within samples, significantly above that of blanks, supports human inhalation as a route of environmental exposure. MPs with dimensions as small as 4 μm but also, surprisingly, >2 mm were identified within all lung region samples, with the majority being fibrous and fragmented. The knowledge that MPs are present in human lung tissues can now direct future cytotoxicity research to investigate any health implications associated with MP inhalation.

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503

504 **Table legend**

505 **Table 1.** Patient and tissue sample information alongside the number of MPs identified within
506 samples by μFTIR spectroscopy. Polymer types and particle characteristics are included, and three
507 different contamination adjustments to display results in units of MP/g of tissue. Abbreviations;
508 PAN=polyacrylonitrile, PE=polyethylene, PES=polyester, PET=polyethylene terephthalate,
509 PMMA=Polymethylmethacrylate, PP=polypropylene, PS=polystyrene,
510 PTFE=polytetrafluoroethylene, PUR=polyurethane, Resin=alkyd/ epoxy/ hydrocarbon,
511 SEBS=styrene-ethylene-butylene co-polymer, TPE=thermoplastic elastomer. R=right lung, L=left
512 lung, Low=lower region of the lung, mid=middle/lingular region of the lung, up=upper region of the
513 lung

514

515 **Figure legends**

516 Fig. 1. Polymer types (A) and shapes (B) of the MPs identified within lung tissue samples.

517 Fig. 2. Images of MPs identified from human lung tissue samples. A, B, C and D= (A=PET) (B=PUR)
518 (C=Resin) (D=PAN). E and F=MPs identified within blanks. (E=PS) (F=PP). Corresponding spectra
519 included in Fig. S2.

520 Fig. 3. Polymer size dimensions and type of each MP identified within (A) human lung tissue samples
521 and (B) 'procedural blank' (triangles) and 'laboratory blank' (circles) samples. Red line represents
522 the assumed inhalable size limit regardless of density.

523 Fig. 4. Particle number (total MPs detected with no account taken for MPs found in controls) and
524 polymer type of MPs identified within human lung tissue samples, assigned to their lung region.

525 Fig. 5. Number (A) and type/quantity (B) of MPs detected in each lung region for individual patients.