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Development of micro- and nanoplastics isolation from blood and organs using reliable digestion protocols and their potential effects on human blood pressure

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List of abbreviations

Polymers

ABS	Acrylonitrile butadiene styrene
ACR	Acrylate polymer
EPDM	Ethylene propylene diene monomer
EVA	Ethylene vinyl acetate
EVOH	Ethylene vinyl alcohol
LDPE	Low density polyethylene
PA	Polyamide = nylon
PA 6 / PA 66	Nylon 6 / Nylon 66
PAN	Polyacrylonitrile
PC	Polycarbonate
PE	Polyethylene
PEAA	Polyethylene-co-acrylic acid
PET	Polyethylene terephthalate
PES	Polyester
PEVA	Polyethylene vinyl acetate
PMMA	Polymethyl methacrylate
POM	Polyoxymethylene
PP	Polypropylene
PS	Polystyrene
PSAN	Polystyrene-co-acrylonitrile
PTFE	Polytetrafluoroethylene
PU	Polyurethane
PVA	Polyvinyl alcohol
PVAc	Polyvinyl propionate/acetate
PVC	Polyvinyl chloride

Identification methods

AFM	Atomic force microscopy
ESI-MS/MS	Electrospray ionization triple-quadrupole mass spectrometer
FTIR	Fourier transform infrared spectroscopy
LDIR	Laser direct infrared spectroscopy
μ-FTIR	Micro fourier transform spectroscopy
μ-Raman	Raman micro-spectroscopy
Pyrolysis GC/MS	Pyrolysis gas chromatography/mass spectrometry
Raman	Raman spectroscopy
TEM	Transmission electron microscopy

Reagents

H_2O_2	Hydrogen peroxide
HNO_3	Nitric oxide
KOH	Potassium hydroxide
NaClO	Sodium hypochlorite
NaOH	Sodium hydroxide

Abstract

The widespread presence of plastics and inadequate waste management have led to the distribution of microplastics and nanoplastics in the environment. Recent studies have shown the presence of microplastics in various human tissues, fluids, and even the bloodstream. However, there is still no standardized, non-particle-damaging method for the digestion of human samples and no clear evidence of the resulting effects on human vascular health in terms of blood pressure.

Therefore, the aim of this dissertation was to develop standardized digestion methods and assessing the potential impact of plastic particles on human blood pressure.

These challenges were addressed by three complementary studies, with the following methodological focus: (1) novel enzymatic method development using pepsin and pancreatin digestion for blood-based microplastic detection, (2) optimization of tissue digestion protocols by testing pepsin and pancreatin as enzymatic reagents and potassium hydroxide as a chemical reagent, and (3) a systematic review of the potential effects of circulating plastic particles on human blood pressure.

The pepsin and pancreatin digestion method was developed to mimic physiological gastrointestinal conditions and proved to be an effective and gentle method for blood digestion, without damaging 5 µm polystyrene particles. While enzymatic methods did not provide sufficient digestion efficiency for porcine organs, digestion with 10 % potassium hydroxide at 37 °C resulted in successful dissolution of lung, liver and kidney organs, and no alterations of 1 and 5 µm polystyrene particles. In investigating the potential physiological relevance of plastic particles in the circulatory system, data from in vitro models, in vivo studies and human clinical trials suggest plausible mechanisms by which particles may cause endothelial injury, platelet activation and inflammation, potentially contributing to blood pressure elevations.

Overall, this thesis highlights the need for standardized, non-destructive digestion protocols that bridge the gap between digestion efficiency and preservation of plastic structure to improve the accuracy of plastic detection in biological samples, to better assess their potential impact on blood pressure.

Zusammenfassung

Die zunehmende Präsenz von Kunststoffen und die einhergehende unzureichende Abfallentsorgung haben zu einer starken Ansammlung von Mikro- und Nanokunststoffen in der Umwelt geführt. Jüngste Studien haben den Nachweis von Mikroplastik in verschiedenen menschlichen Geweben, Flüssigkeiten und sogar im Blutkreislauf bestätigt. Es gibt jedoch noch immer keine standardisierte, partikelschonende Methode für den Verdau menschlicher Proben und keine eindeutigen Belege für die daraus resultierenden Auswirkungen auf die menschliche Gefäßgesundheit in Bezug auf den Blutdruck.

Ziel dieser Dissertation war es daher, standardisierte Verdaumethoden zu entwickeln und die möglichen Auswirkungen von Kunststoffpartikeln auf den menschlichen Blutdruck zu bewerten.

Diese Herausforderungen wurden durch drei ergänzende Studien mit folgenden methodischen Schwerpunkten behandelt: (1) Entwicklung einer neuen Verdaumethode unter Verwendung von Pepsin und Pankreatin für den Nachweis von Mikroplastik im Blut, (2) Optimierung von Protokollen für den Gewebeverdau mittels Pepsin und Pankreatin als enzymatische Reagenzien und Kaliumhydroxid als chemisches Reagenz und (3) eine systematische Untersuchung der potenziellen Auswirkungen zirkulierender Plastikpartikel auf den menschlichen Blutdruck.

Die Verdauungsmethode mit Pepsin und Pankreatin wurde entwickelt, um physiologische Magen-Darm-Bedingungen nachzuahmen, und hat sich als wirksame und schonende Methode für den Blutverdau erwiesen, ohne 5 µm große Polystyrolpartikel zu beschädigen. Während enzymatische Methoden keine ausreichende Verdauungseffizienz für Schweineorgane lieferten, führte die Verdauung mit 10 % Kaliumhydroxid bei 37 °C zu einer erfolgreichen Auflösung von Lungen-, Leber- und Nierenorganen, ohne dass 1 und 5 µm großen Polystyrolpartikel verändert wurden. Bei der Untersuchung der potenziellen physiologischen Relevanz von Kunststoffpartikeln im Kreislaufsystem zeigten Daten aus In-vitro-Modellen, In-vivo-Studien und klinischen Studien am Menschen, dass Partikel zu

Endothelverletzung, Thrombozytenaktivierung und Entzündungen führen und damit möglicherweise zu einer Erhöhung des Blutdrucks beitragen können.

Insgesamt unterstreicht diese Arbeit den Bedarf an standardisierten, zerstörungsfreien Verdauungsprotokollen, die die Lücke zwischen Verdauungseffizienz und Erhaltung der Kunststoffstruktur schließen, um die Genauigkeit des Nachweises von Kunststoffen in biologischen Proben zu verbessern und ihre potenziellen Auswirkungen auf den Blutdruck besser beurteilen zu können.

1 Introduction

1.1 Growing concern of plastic pollution: Distribution and classification

The widespread presence of microplastics (MPs) and nanoplastics (NPs) in the environment has raised increasing concern about their potential impact on human health in the last couple of years (World Health Organization, 2022). A critical issue concerning plastic is its longevity within the environment, where it persists over a timespan ranging from hundreds to thousands of years to complete dissolution (Jambeck et al., 2015). A growing focus on sustainable materials has led to the development of biodegradable plastics as potential alternatives to conventional polymers. However, despite their intended environmental benefits, materials such as polylactic acid (PLA) and polyhydroxyalkanoates (PHAs) face significant challenges in terms of stability, biodegradability and regulatory standards, and typically struggle with brittleness, heat resistance, colour changes, low cost solutions and insufficient degradation in natural environments (Lara-Topete et al., 2024). Consequently, despite growing environmental concerns, plastic polymers continue to be an integral part of various industrial and consumer goods due to their unique functional properties.

Therefore, given this widespread presence, it is crucial to classify these particles according to their origin and formation process. Furthermore, it requires a clear identification of physical forms such as fibres, particles, fragments, and films, along with a precise and standardized nomenclature for MPs and NPs, to effectively assess their potential health risks (Figure 1). Plastic particles are divided into primary MPs, which are intentionally produced in microscopic size for use in personal care products or pharmaceuticals. Secondary MPs are formed unintentionally through the degradation of larger plastic waste in the environment via physical, chemical, and biological processes, such as thermal or photo-oxidative degradation processes, or plastic waste generated in wear and tear from means of transport, washing of synthetic fabrics or the degradation of plastic materials in urban environments (Da Silva Antunes et al., 2025; Morreale & La Mantia, 2024; Song et al., 2024).

Scientific literature provides a variety of definitions for MPs and NPs, resulting in different classifications with regard to their size. In general, MPs are considered to be plastic

fragments ranges from 100 nm to 5 mm (Figure 1) (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2016). In contrast, NPs are frequently characterised as particles in the nanometre range (Figure 1). Some definitions classify NPs as ranging from 1 to 100 nm (Ivleva, 2021), while others extend the range from 1 nm to 1 µm (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2016). Another category is that of sub-microplastic, which describes particles in the size range between 100 nm and 1 µm (Figure 1) (Ivleva, 2021; Vasudev et al., 2015). In the following thesis, particles up to a size of 100 nm are referred to as NPs and those above this size are referred to as MPs.

The accumulation of plastics in the environment has led to the identification of the most prevalent types, which include polystyrene (PS), polypropylene (PP), polyvinyl chloride (PVC), polyethylene (PE), polyethylene terephthalate (PET), polyamide (PA), polymethyl methacrylate (PMMA), polyester (PES), polyurethane (PU) and polyacrylate (PAC). Fibres and fragments are the dominant forms, while films, sheets, and spheres appear less frequently (Figure 1) (Khare & Khare, 2023; Kutralam-Muniasamy et al., 2022; Vdovchenko & Resmini, 2024). The discrepancy in the reported prevalence of MPs across diverse literature sources can be ascribed to variations in the size detection thresholds. This underscores the necessity for standardised research methodologies to facilitate a more precise evaluation of smaller MPs and their potential health effects (Vdovchenko & Resmini, 2024). These polymers come from various widely used consumer and industrial applications, such as PET in water bottles, PP in yoghurt packaging, PS in food containers and packaging materials, PE for packaging and foils, PVC in construction or PES in clothing (Figure 1) (Desidery & Lanotte, 2022; Seewoo et al., 2024).

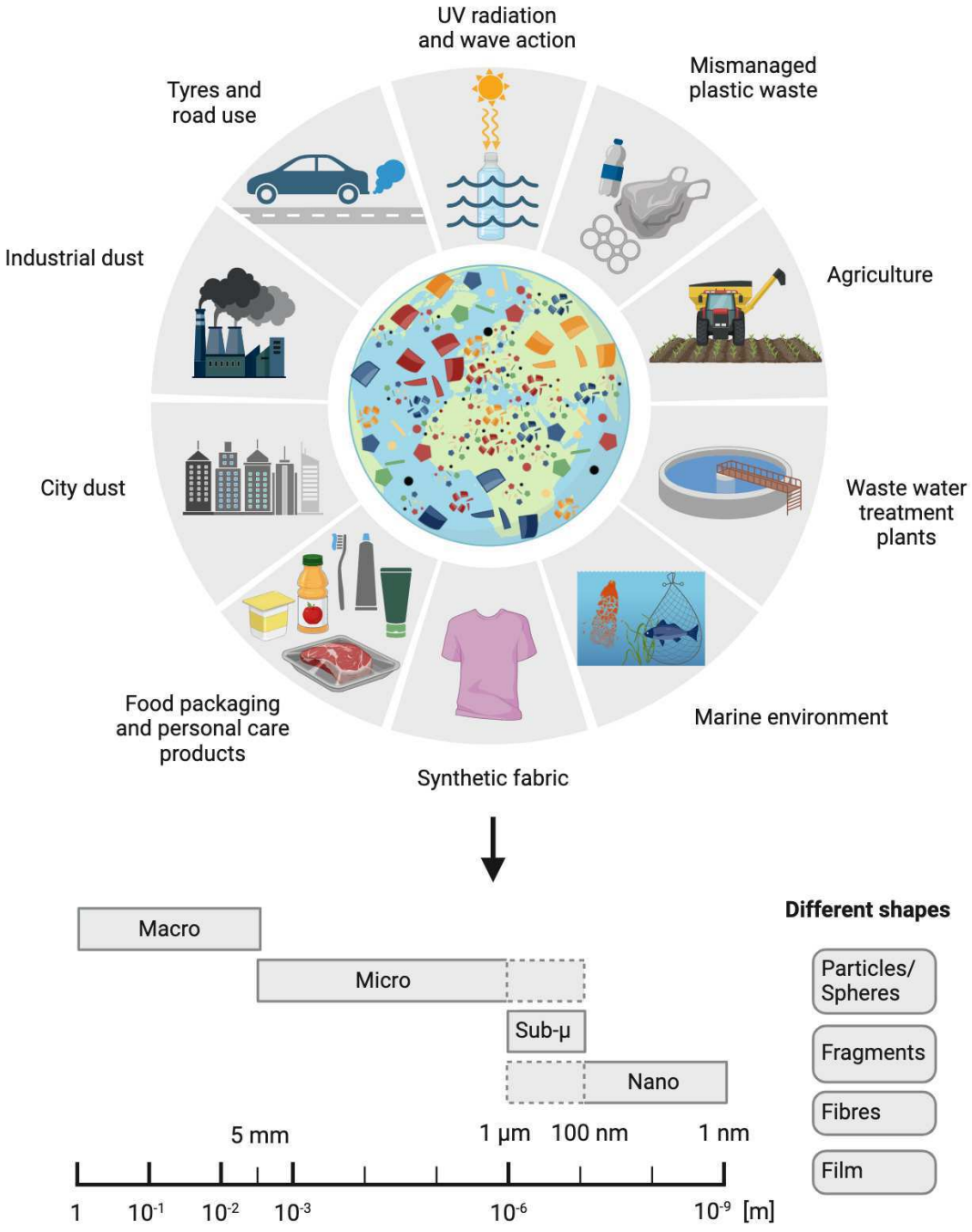

Figure 1. Environmental sources and characteristics of primary and secondary plastics. Comprehensive overview of the global spread of plastic particles, surrounded by key anthropogenic sources and size classification of MPs and NPs that occur in various morphological forms (Created in BioRender. Geppner, L. (2025) https://BioRender.com/h99x774).

1.2 Analytical workflow for detection of plastic particles in human samples

Considering the rising number of studies identifying MPs in human organs, fluids and tissues, the development of standardised and validated digestion methods is essential for assuring the comparability and reliability of these findings. However, there are currently no established standardized methods for the collection, preparation, quantification and characterization of plastic particles.

A variety of methods have been utilized for the purpose of detecting plastics in the context of the digestion of human body fluids, organs and tissues (Figure 2). Among these methods, chemical digestion stands out as a prominent approach. The most commonly used alkaline digestive agent is potassium hydroxide (KOH). However, even this is not a standardized process, as it is used in different concentrations (1 %, 10 %, 15 %, 30 %), for extremely different durations, which can range from a few hours to weeks, and in different incubation temperatures, which range from room temperature to 135 °C (Dzierżyński et al., 2024; Yun et al., 2024; Zhang et al., 2021a). However, sodium hydroxide (NaOH) is also used, in addition to acids such as nitric acid (HNO_3) and oxidizing agents such as hydrogen peroxide (H_2O_2) and Fenton's reagent (H_2O_2 with iron(II) sulfate) (Dzierżyński et al., 2024). Alkaline digestion methods are generally considered to be a beneficial approach for the degradation of organic materials. However, it has been observed that the use of 10 % KOH and 10 M NaOH solutions can result in alterations to the physical characteristics of cellulose acetate and PET, including changes in shape and size (Dehaut et al., 2016), showed reduction of the recovery rate of PVC, and color changes of PA 66 (Karami et al., 2017). Acidic digestion revealed good removement of organic matter in a short time by HNO_3 but caused the ABS, PA, and PET particles to decompose or disintegrate into smaller fragments (Scheurer & Bigalke, 2018). Additionally, it was reported that HNO_3 can destroy PET, PS, PP, PU, PE, PVC, PE and PA (López-Rosales et al., 2021). Results of H_2O_2 digestion revealed satisfactory digestion efficacy above 50 °C, but created foamy and oxidized particles, degraded PA 6 and PA 66 and changed the color of PET (Karami et al., 2017).

In a limited number of cases, an enzymatic method employing proteinase K (Brits et al., 2024; Leslie et al., 2022) or Corolase (Amato-Lourenço et al., 2021, 2024) were used for the digestion of human blood, lung tissue, or tissue from the olfactory bulb. A study by Cole and colleagues revealed proteinase K as the most effective method for releasing microplastic particles from zooplankton samples (Cole et al., 2014). In addition, digestion of seabird stomachs with Corolase was reported to be more challenging due to the high cost and limited availability of this reagent (Lavers et al., 2019).

The selection of an appropriate detection method for MPs involves a careful evaluation of factors such as resolution, cost, and accessibility. Each technique possesses unique strengths and limitations, necessitating a judicious choice to achieve optimal outcomes. A lot of different identification techniques have been used for the detection of plastic particles in human samples (Figure 2).

These include **Raman micro-spectroscopy** (μ-Raman), a highly prevalent identification method, which combines light microscopy with Raman spectroscopy. It possesses a high degree of spatial resolution and provides information about MPs ranging from 1 μm to a few mm (Figure 2). However, it is only applicable to a small amount of sample, extensive sample pre-treatment is required, only applicable to small areas and very time-consuming (Dzierżyński et al., 2024; Jahanpeyma & Baranya, 2024). Conversely, **pyrolysis gas chromatography/mass spectrometry** (pyrolysis GC/MS) is used to report particle masses per polymer type and is particularly suitable for small sample quantities. Unlike spectroscopic methods, pyrolysis GC/MS allows the identification of very small plastic particles (Figure 2), but the method is limited by the preceding sample filtration step. As a result, detection in blood samples using pyrolysis GC/MS has been limited to particles larger than 0.7 μm (Leslie et al., 2022) or 0.3 μm (Brits et al., 2024), depending on the filter pore size used during isolation of MPs. In addition, its efficiency is reduced at high concentrations of impurities, it is destructive to the sample and dry samples are required. While it can detect the chemical composition and concentration of plastic-type substances, it remains incapable of quantifying

the number or shape of particles (Sharma et al., 2024; Xu et al., 2023). Conversely, **scanning electron microscopy** (SEM) offers a higher resolution limit of 1 to 10 nm (Figure 2), but its application is limited to the quantification of the most important elements in plastics, so that additional confirmation of the types of plastic is required. This process is both time-consuming and expensive (Zhu et al., 2024). An efficient, simple, and non-destructive detection technique that can determine the material type (plastic or non-plastic) is **micro fourier transform infrared spectroscopy** (μ-FTIR) and combines optical imaging with chemical information from infrared spectra. This technique is non-destructive and less expensive than Raman or pyrolysis GC/MS, with detection limit down to 1 μm (Figure 2). But it shows strong interference with water and is not suitable for the analysis of NPs (Dzierżyński et al., 2024; Jahanpeyma & Baranya, 2024). **Laser direct infrared spectroscopy** (LDIR) is similar to μ-FTIR and can determine particle number, size and morphology. The spectrometer can identify the polymer type and detects small MPs from 20 to 5000 μm (Figure 2) (Ghanadi et al., 2024).

One of the major challenges in the identification of MPs is the presence of polymer mixtures. It is difficult to establish a standardized analysis procedure, due to a variety of formulas and origins of polymers, since many plastic products are composed of blends of different polymers and additives, which can complicate their chemical characterization. Traditional spectroscopic methods like FTIR and Raman are optimized for identifying pure polymers, but when plastics are mixed or degraded, their spectral signatures overlap, making precise identification difficult (Yun et al., 2024). As a solution, thermogravimetric analyzer coupled with a fourier transform infrared (TG-FTIR) and thermal extraction-desorption gas chromatography-mass spectrometry (TED-GC-MS) provide strong advantages in the identification of mixed microplastics through different discrimination mechanisms and identifies particles of any size (Cho et al., 2023).

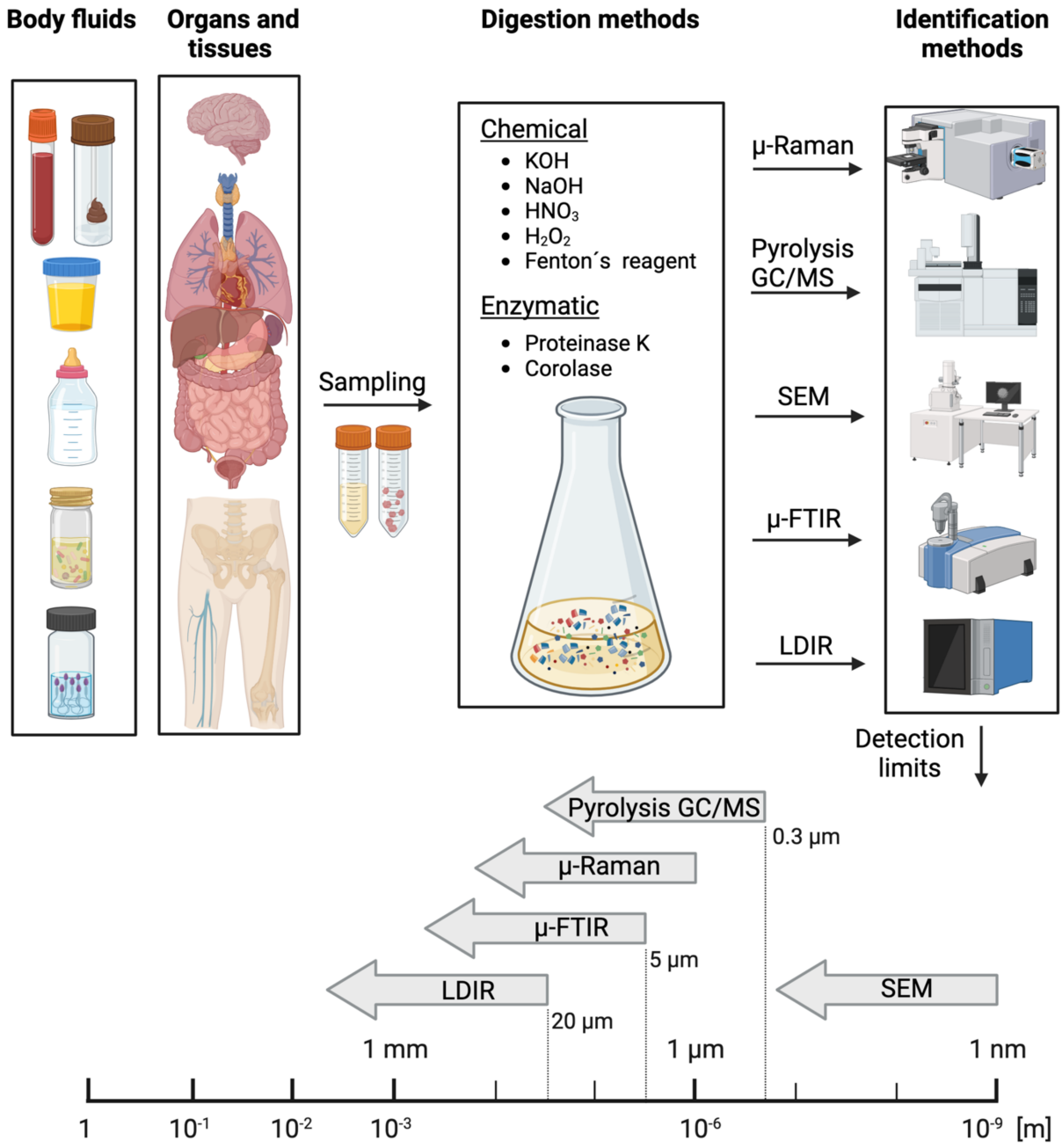

Figure 2. Analytical workflow for the detection of plastic particles in human samples. Overview of different human matrices, digestion methods, and identification techniques with the corresponding lower detection limits for MPs and NPs detection (Created in BioRender. Geppner, L. (2025) https://BioRender.com/r75r380).

1.3 Evidence of plastic particles in the human body

A number of studies have demonstrated that MPs have the capacity to enter the human body via various routes of exposure, most notably through inhalation or digestion (Figure 3). These particles are omnipresent in the environment and can be ingested by consuming contaminated foodstuffs, such as seafood or by inhaling air contaminated with MPs (Belmaker et al., 2024; Emenike et al., 2023; Waring et al., 2018; Yuan et al., 2022).

Data on sampling examinations, surgical operations, diet scenarios were obtained to investigate MPs in different human body fluids, such as blood, breast milk, saliva, stomach content, urine, faeces and seminal fluid (Table 1).

When examining the presence of MPs in **human blood**, a diverse range of polymer types and particle sizes has been identified, indicating the complexity of their systemic distribution and potential physiological interactions. The first detection of plastic particles, in conjunction with the implementation of an enzymatic proteinase K digestion demonstrated a concentration of 1.6 µg/mL and a size greater than 0.7 µm, by using pyrolysis GC/MS. These findings were reported by Leslie and colleagues in their study of 22 human blood samples (Leslie et al., 2022). Comparable outcomes, utilizing the same techniques, were attained with a threefold increase in the number of test subjects, detecting MPs ranging in size from 0.3 to 500 µm at a similar concentration of 1.07 µg/mL (Brits et al., 2024). In a further comparable study, in which Raman was also employed, the presence of plastic was detected in a concentration of 2.2 ± 1.42 MPs/ml with a size range of 10 to 65 µm (Xu et al., 2024). Guan and colleagues analysed human blood samples from eight participants after digestion with trypsin and a bleach solution of sodium hypochlorite (NaClO) and detected MPs ranging in size from 19.92 to 66.73 µm using µ-Raman (Guan et al., 2023). Blood tests also revealed changes following a cardiac surgical procedure, after chemical digestion with 68 % HNO_3 and LDIR as a detection method. It was found that before surgery, mainly PET with a size of 30 to 50 µm was detected, while after surgery, more PA with a size of 20 to 30 µm was found (Yang et al., 2023). The study by Leonard and colleagues is of particular interest because, whilst it

states that MPs are present in a concentration that is similar to those previously mentioned in the literature (1.84 – 4.65 µg/mL), it detects particles with a size of up to 3 mm. A mix of enzymatic and chemical digestion methods was applied using pancreatin, lipase and 30 % H_2O_2 followed by µFTIR to identify plastic particles (Leonard et al., 2024). The predominant types of plastic detected in all of these blood analyses were PE, PET, PS, PA and PMMA.

However, the presence of plastic particles has been detected not only in blood, but also in other body fluids. The first discovery of MPs in **breast milk** was made in a study by Ragusa and colleagues. By using 10 % KOH for digestion and subsequent analysis with Raman microspectroscopy, MPs were found in 26 out of 34 samples, with sizes ranging from 2 to 12 µm. This is of great concern, since it has an impact on the extremely vulnerable group of infants (Ragusa et al., 2022). A methodologically comparable study of 59 breast milk samples revealed the presence of MPs in 39 of these samples (Saraluck et al., 2024). The most prevalent types of MPs identified were PP, PE and PVC. The study additionally established a correlation between the presence of MPs and the development of maternal hygiene practices and the occurrence of breastfeeding complications (Saraluck et al., 2024). A concentration of 20.2 MPs/g, with a particle size ranging from 20 µm to 500 µm, was identified using 68 % HNO_3 for digestion and LDIR for particle identification, which concluded that breastfeeding and the utilisation of feeding bottles may be potential sources of exposure for infants (Liu et al., 2023).

Furthermore, in **saliva** and **stomach content**, connected components of the digestive system, MPs were discovered. The size range of the 22 saliva samples was found to be 44.67 to 210.64 µm (Huang et al., 2022), while the 26 stomach content samples exhibited a significantly larger size range of 802.1 ± 858.6 µm (Özsoy et al., 2024). The methodological differences between these studies are that the saliva was digested with 68 % HNO_3 and identified by µ-FTIR and LDIR, while the stomach content was digested using 30 % KOH:NaClO and MPs were detected by µ-Raman (Huang et al., 2022; Özsoy et al., 2024).

For the detection of MPs in **urine**, two studies have achieved very similar results by using the same methods. By means of chemical digestion with 10 % KOH and detection by µ-

Raman, MPs measuring between 3 and 15 µm were identified (Massardo et al., 2024; Pironti et al., 2022).

A large number of studies have also investigated the detection of MPs in the human **faeces** of adults and children, but also in the meconium of newborns (Ho et al., 2022; Liu et al., 2023; Schwabl et al., 2019; Wibowo et al., 2021; Zhang et al., 2021a). The earliest detection of plastic in the faeces of 8 adults was detected by FTIR, which revealed the presence of 2 MPs/g with a size ranging from 50 to 500 µm (Schwabl et al., 2019). Further investigations used Raman as a detection method after successful digestion using 10 % KOH + 30 % H_2O_2 or Fenton's reagent respectively and mainly detected PP, PS, PE and PET (Ho et al., 2022; Wibowo et al., 2021). In this case even at least 10 times the amount of particles, namely 20.4 to 138.9 MPs/g, could be detected, compared to the findings by Schwabl and colleagues (Ho et al., 2022). In the analysis of PET and PC levels in stool samples from infants and adults, which were digested with 1 % KOH and examined by ESI-MS/MS, infant subjects exhibited a higher median PET value measuring 36,000 ng/g and a slightly lower value of 78 ng/g for PC, in comparison to adults, who exhibited a value of 2600 ng/g and 110 ng/g, respectively (Zhang et al., 2021a). A comparison between meconium from newborns and infant faeces, which have been digested using 68 % HNO_3 and quantified using LDIR, shows that the number of particles in newborns (51.5 MPs/g) is almost twice that in infants (26.6 MPs/g) (Liu et al., 2023). The amount of particles after different scenarios of plastic use and food consumption in faeces was investigated in an intervention study with 15 volunteers. The stool samples were digested with 30 % H_2O_2 and the number of MPs was determined using FTIR. The results showed median amount of MPs after a normal diet of 4.4 MPs/g, after a low-plastic diet 4.3 MPs/g, after the reset phase 3.5 MPs/g and after a high-plastic diet 2.7 MPs/g of particles in a size range between 50 and 500 µm were found (Hartmann et al., 2024).

Furthermore, a discrepancy in the frequency of plastic particles has been demonstrated to be associated with the severity of disease. Participants diagnosed with inflammatory bowel disease (IBD) showed significantly more plastic particles in stool samples (41.8 MPs/g)

compared to those classified as healthy (28 MPs/g), suggesting that MPs exposure is related to the disease process or that IBD exacerbates MPs retention (Yan et al., 2022).

MPs have also been found in **seminal fluid**. Using pyrolysis GC/MS, PVC was detected in 5 samples at a concentration of 15.34 ± 23.31 µg/mL and LDIR in 25 samples revealed a variety of polymers with a size of 96.19 ± 74.17 µm at a concentration of 0.23 ± 0.45 MPs/mL (Zhao et al., 2023). Almost at the same time, another study was published that used µ-Raman to find much smaller particles, specifically in a size of 2 to 6 µm, in human seminal fluid (Montano et al., 2023).

In addition to the detection of MPs in human body fluids, a number of studies have identified MPs in various organ systems, including the cardiovascular, nervous, respiratory, hepatic, renal, gastrointestinal, reproductive, and skeletal systems (Table 2).

Investigations of the **cardiovascular system** have shown, for example, that MPs can accumulate in various areas of the heart. With the help of LDIR, mainly PET and PU of a size of 20 to 469 µm were found in pericardia, epicardial adipose tissues, pericardial adipose tissues, myocardia and left atrial appendages (Yang et al., 2023). However, an accumulation of particles could also be detected in atherosclerotic plaque using pyrolysis GC/MS (Liu et al., 2024). After digestion using 68 % HNO_3, 118.66 ± 53.87 µg/g of different polymers were detected in one study (Liu et al., 2024), whereas Marfella and colleagues detected an amount of 21.7 ± 24.5 µg/mg PE in plaques of 312 patients (Marfella et al., 2024). Additionally, LDIR analysis of 26 thrombosis samples revealed an average concentration of 91.1 µg/g of MPs ranging in size from 20 to 50 µm (Wang et al., 2024). In a further investigation, the presence of small MPs with a size of about 1 to 6 µm was detected, however, their identification was restricted to one individual particle (Wu et al., 2023). Furthermore, tissue samples of the human saphenous vein digested with 30 % H_2O_2 were used for the detection of 16 to 1074 µm MPs at a concentration of 14.9 ± 17.2 MPs/g using µFTIR (Rotchell et al., 2023).

Very recent investigations were also able to detect MPs in the **nervous system** for the first time. Using KOH digestion, followed by pyrolysis GC/MS, almost 1.5 times the amount of

MPs was found in the brain in 2024 (4917 µg/g) compared to 2016 (Nihart et al., 2025). A further study was conducted in which the olfactory bulb from 15 patients was analysed using µ-FTIR and detected 1 to 4 MPs/sample with a size ranging from 3 to 26.4 µm (Amato-Lourenço et al., 2024).

As demonstrated in an investigation conducted in 1998, there is compelling evidence of the presence of fibres in the **respiratory system**. This study involved the examination of 114 human lung specimens, of which 87 % exhibited the presence of cellulosic and plastic fibres (Pauly et al., 1998). A much more recent study was then able to detect 31 MPs with a size between 1.6 and 5.56 µm in 13 of the 20 samples using improved identification methods such as light microscopy or Raman (Amato-Lourenço et al., 2021). Furthermore, the utilisation of µ-FTIR facilitated the identification of 0.69 ± 0.84 MPs/g (Jenner et al., 2022).

In the **hepatic system**, MPs could be detected in the liver using µ-Raman. A total of 4.6 MPs/g was found in 6 samples with a size of 3.0 to 29.5 µm (Horvatits et al., 2022). Nihart and colleagues conducted a comparative analysis of MPs in the liver and **renal system** (kidneys) between 2016 and 2024, employing methodologies similar to those utilised in brain tissue analysis. The study revealed a fourfold increase in liver MPs in 2024 (433 µg/g), while the kidney level remained consistently high at approximately 400 µg/g of MPs over the eight-year period. In both cases, the types of plastic detected were found to be relatively unchanged, with PE consistently being the most prevalent type (Nihart et al., 2025). A size range of particles, from 1 to 29 µm, could also be determined in the kidney using µ-Raman (Massardo et al., 2024).

To the best of our knowledge, only one study so far has detected plastic particles in the **gastrointestinal tract**. For this purpose, 11 colectomy samples were digested by KOH and identified by µ-FTIR MPs, resulting in the identification of very large particles in the range of 0.8 to 1.6 mm with a concentration of 28.1 ± 15.4 MPs/g (Ibrahim et al., 2021).

The presence of MPs in the female **reproductive system** has been demonstrated by a substantial number of studies, primarily due to the ethical acceptability of the placenta as a research organ. Unlike other organs, the placenta can be removed after birth without the need

for invasive procedures. The digestion methods used here were very standardised, as all samples were chemically digested, with 10 % KOH being used for the majority. Ragusa and colleagues were the first to detect 5 to 10 µm of pigmented MPs using µ-Raman. Notably, five MPs were identified in the foetal side of the placenta, underscoring the potential for foetal exposure to MPs (Ragusa et al., 2021). This was followed by another study using µFTIR, but only one placenta was analysed and no concentration was reported, only the fact that PE, PP and PU were found in a size larger than 50 µm (Braun et al., 2021). In two further placenta studies, the LDIR analysis method was able to analyse particles in a similar size range of 20 to 500 µm particles with an average frequency of 18 MPs/g (Liu et al., 2023) or 2.7 ± 2.65 MPs/g between 20.34 and 307.29 µm (Zhu et al., 2023) respectively. An MPs analysis in a 15-year period showed that in placenta both the particle quantity and size increased continuously from 2006 (1 to 8 µm of 4.1 ± 1.3 MPs/50g) to 2013 (1 to 17 µm of 7.1 ± 0.9 MPs/50g) and again to 2021 (1 to 44 µm of 15.5 ± 3 MPs/50g). While the three most common polymers in 2006 were PP, PE and PVC, in 2021 it was PES, PET and PVA (Weingrill et al., 2023). In 2024, two large-scale studies on the detection of MPs in placenta were published. In one study, 62 tissues were analysed using FTIR and pyrolysis GC/MS, in which 126.8 ± 137.5 mg/g of MPs larger than 1 µm were found (Garcia et al., 2024). In a separate study, 50 MPs were identified in 31 out of 50 samples, with a size range of 1.03 to 6.84 µm using µ-Raman (Yun et al., 2024). In the male reproductive organ, MPs could be detected in 6 testes samples using LDIR, with particles ranging from 20 to 100 µm in size and a concentration of 11.6 ± 15.52 MPs/g (Zhao et al., 2023).

Finally, evidence of MPs has been found in the **skeletal system**. After digestion with H_2O_2 and analysis by µFTIR and µ-Raman, 11 to 151 µm particles with a concentration of 6.35 ± 1.95 and 4.79 ± 1.92 were found in 45 hip and knee synovium, respectively (Li et al., 2024). Thereby, the small vessels of the hip joint capsule, with a slower flow velocity, could lead to the larger number of MPs compared to the synovium (Grieshaber-Bouyer et al., 2019; Li et al., 2024). Furthermore, MPs were examined in eight intervertebral discs, bones, and cartilages after digestion with Fenton's reagent and subsequent µ-Raman analysis. The smallest

particles, with a size of 87.5 ± 30.7 μm, were found in the cartilages, while the largest, with a size of 159.5 ± 103.8 μm, were found in the intervertebral disc, where also the largest concentration of 61.1 ± 44.2 MPs/g was measured. Bones and cartilages exhibited significantly lower concentrations, with the lowest observed in the bones, measuring 22.9 ± 15.7 MPs/g (Yang et al., 2025). A possible reason for the increased deposition of MPs in the intervertebral disc is that the composition of the disc creates a closed environment that hinders the excretion of substances. In contrast, the blood supply to the bones is provided by the periosteum, which contains a dense network of blood vessels (Yang et al., 2025).

Table 1. Summary of detected microplastics in human body fluids.

Human body fluids	Particle properties found	Concentration found	Investigated number (n)	Digestion Method	Identification method	References (sorted by date)
Blood	0.7 – 500 µm PET > PE > PS > PMMA	1.6 µg/mL	22	Proteinase K	Py-GC/MS	(Leslie et al., 2022)
	19.92 – 66.73 µm LDPE, PEAA, PA, PSAN and PVA	6 MPs	8	0.25 % trypsin + 6-14 % NaClO	µ-Raman	(Guan et al., 2023)
	30 – 50 µm PET (67 %) 20 – 30 µm PA (57 %)	–	7 presurgery 7 postsurgery	68 % HNO_3	LDIR	(Yang et al., 2023)
	~10 – 65 µm PE, PE-co-PP, PP (58 %)	2.2 ± 1.42 MPs/mL	15	Proteinase K	Raman, Py-GC/MS	(Xu et al., 2024)
	7 – 3000 µm PE, EPDM, EVA/EVOH, PA and PS (70 %)	2.47 ± 4.17 MPs/mL 1.84 – 4.65 µg/mL	20	Pancreatin + Lipase + 30 % H_2O_2	µ-FTIR	(Leonard et al., 2024)
	0.3 – 500 µm PE > PVC > PET > PMMA	1.07 µg/mL	68	Proteinase K	Py-GC/MS	(Brits et al., 2024)
Breast milk	2 – 12 µm PE > PVC > PP found in 76 % of the samples	–	34	10 % KOH	µ-Raman	(Ragusa et al., 2022)
	20 – 500 µm PU > PA > PE (87 %)	20.2 MPs/g	18	68 % HNO_3	LDIR	(Liu et al., 2023)
	MPs found in 39 % of the samples (PP, PE, PVC)	–	59	10 % KOH	µ-Raman	(Saraluck et al., 2024)
Saliva	44.67−210.64 µm PU, PES, PE (68 %) found in 68 % of the samples	4 MPs/mL	22	68 % HNO_3	µ-FTIR, LDIR	(Huang et al., 2022)
Stomach content	802.1 ± 858.6 µm PE > PP > PMMA > PA > PET (80 %)	9.4 ± 10.4 MPs/individuum	26	30 % KOH:NaClO	µ-Raman	(Özsoy et al., 2024)
Urine	4 – 15 µm PP > PE > PVC (100 %)	7 MPs	6	10 % KOH	µ-Raman	(Pironti et al., 2022)
	3 – 13 µm PE > PS	23 polymers + pigments	10	10 % KOH	µ-Raman	(Massardo et al., 2024)

Table 1. Continued

Human body fluids	Particle properties found	Concentration found	Investigated number (n)	Digestion Method	Identification method	References (sorted by date)
Faeces	50 – 500 µm PP > PET (80 %)	2 MPs/g	8	Chemical	µ-FTIR	(Schwabl et al., 2019)
	20 – 800 µm PP > PET > PS	1 – 36 MPs/g	24 students	30 % H_2O_2	µ-FTIR	(Zhang et al., 2021b)
	PET PC	36000 ng/g 78 ng/g	6 infant	1 % KOH + 1-pentanol	ESI-MS/MS	(Zhang et al., 2021a)
	PET PC	2600 ng/g 110 ng/g	10 adults			
	PP > PE > PET > PS (100 %)	6.94 – 16.55 µg/g	11	Fenton´s reagent	Raman	(Wibowo et al., 2021)
	4.4 – 333.2 µm PET > PA > PP > PE > PC (54 %)	28 MPs/g	50 healthy	30 % H_2O_2 + 65 % HNO_3	µ-Raman	(Yan et al., 2022)
	1.7 – 393.8 µm PET > PA > PP > PE > PC (66 %)	41.8 MPs/g	52 IBD			
	40.2 – 4812.9 µm PS > PP > PE > PET > PVC (100 %)	20.4 – 138.9 MPs/g	8	10 % KOH + 30 % H_2O_2	Raman	(Ho et al., 2022)
	20 – 500 µm PA > PU (83 %)	51.5 MPs/g	12 newborn	68 % HNO_3	LDIR	(Liu et al., 2023)
	20 – 500 µm PA > PU (76 %)	26.6 MPs/g	12 infant			
	50 – 500 µm PE > PET > PA > PP > PS	4.4 MPs/g	15 normal diet	30 % H_2O_2	FTIR	(Hartmann et al., 2024)
		4.3 MPs/g	15 low plastic diet			
		3.5 MPs/g	15 reset phase			
		2.7 MPs/g	15 high plastic diet			
Seminal fluid	96.19 ± 74.17 µm PVC > PE > PA > PS > PP > PET (75 %)	0.23 ± 0.45 MPs/mL	25	10 % KOH	LDIR	(Zhao et al., 2023)
	PVC (98.1 %)	15.34 ± 23.31 µg/mL	5	10 % KOH	Py-GC/MS	
	2 – 6 µm PP, PS, PET, PE, POM, PVC, PC	16 MPs in 6 samples	10	10 % KOH	µ-Raman	(Montano et al., 2023)

Table 2. Summary of detected microplastics in human organ and tissue samples.

Human organs & tissues	Particle properties found	Concentration found	Investigated number (n)	Digestion Method	Identification method	References (sorted by date)
Heart	20 – 469 µm PET, PU (90 %)	–	15	30 % H_2O_2 + 68 % HNO_3 + 10 % NaOH	LDIR	(Yang et al., 2023)
Atherosclerotic plaque	PET, PA-66, PVC and PE	118.66 ± 53.87 µg/g	17	68 % HNO_3	Py-GC/MS	(Liu et al., 2024)
	< 1 µm PE < 1 µm PVC	21700 ± 24500 µg/g 5200 ± 2400 µg/g	312	–	Py-GC/MS, TEM	(Marfella et al., 2024)
Thrombi	1 ~ 6 µm LDPE	1 MPs	26	30 % KOH	Raman	(Wu et al., 2023)
	20 – 50 µm PE > ACR > PP > CPE > PMMA > PU (94 %)	61.75 µg/g in IS thrombi, 141.8 µg/g in MI thrombi, 69.62 µg/g in DVT	30	68 % HNO_3	LDIR	(Wang et al., 2024)
Saphenous vein tissue	16 – 1074 µm Alkyd resin, PVAc, PA-EVA (85 %)	14.9 ± 17.2 MPs/g	5	30 % H_2O_2	µ-FTIR	(Rotchell et al., 2023)
Brain	PE > PP PE > PP > PVC	3345 µg/g 4917 µg/g	28 (2016) 28 (2024)	10 % KOH	Py-GC/MS	(Nihart et al., 2025)
Olfactory bulb	3 – 26.4 µm PP > PA > PEVA (56 %)	1 – 4 MPs/sample	15	Corolase® 7089	µ-FTIR	(Amato-Lourenço et al., 2024)

Table 2. Continued

Human organs & tissues	Particle properties found	Concentration found	Investigated number (n)	Digestion Method	Identification method	References (sorted by date)
Lung	–	83 % contained fibres 97 % contained fibres	81 nonneoplastic tissue 33 cancerous tissue	–	microscopic	(Pauly et al., 1998)
	1.60 – 16.8 µm PP > PE > PVC (46 %)	37 MPs in 13 samples	20	Corolase® 7089	Light microscopy, Raman	(Amato-Lourenço et al., 2021)
	PP > PET > Resin > PE > PTFE > PS (84 %)	0.69 ± 0.84 MPs/g	11	30 % H_2O_2	µ-FTIR	(Jenner et al., 2022)
Liver	3.0 – 29.5 µm PS > PVC > PET (78 %)	4.6 MPs/g	6	40.5 % KOH + 6-14 % NaClO + 30 % H_2O_2	µ-Raman	(Horvatits et al., 2022)
	PE > PA PE > PP > PA	~100 µg/g 433 µg/g	27 (2016) 23 (2024)	10 % KOH	Py-GC/MS	(Nihart et al., 2025)
Kidney	PE > PVC > PA PE > PP	~400 µg/g 404 µg/g	23 (2016) 20 (2024)	10 % KOH	Py-GC/MS	(Nihart et al., 2025)
	1 – 29 µm PE > PS	43 polymers + pigments	10	10 % KOH	µ-Raman	(Massardo et al., 2024)
Colon	0.8 – 1.6 mm PC > PA > PP	28.1 ± 15.4 MPs/g	11	10 % KOH	µ-FTIR	(Ibrahim et al., 2021)

Table 2. Continued

Human organs & tissues	Particle properties found	Concentration found	Investigated number (n)	Digestion Method	Identification method	References (sorted by date)
Placenta	~5 – 10 µm pigmented MPs	5 MPs in foetal side 4 MPs in maternal side 3 in chorioamniotic membranes	6	10 % KOH	µ-Raman	(Ragusa et al., 2021)
	> 50 µm PE, PP, PU	–	1	chemical	µ-FTIR	(Braun et al., 2021)
	20 – 500 µm PU > PA > PE (90 %)	18 MPs/g	18	68 % HNO_3	LDIR	(Liu et al., 2023)
	1 – 8 µm PP, PE > PVC > PU (77 %) 1 – 17 µm PP > PET, PVA, PES > ABS > PE, PU, PC (71 %) 1 – 44 µm PES > PET, PVA > PP > PE > PVC > PU, PC (73 %)	4.1 ± 1.3 MPs/50 g 7.1 ± 0.9 MPs/50 g 15.5 ± 3.0 MPs/50 g	10 (2006) 10 (2013) 10 (2021)	10 % KOH	AFM, Raman	(Weingrill et al., 2023)
	20.34 – 307.29 µm PVC > PP > PET (65 %)	2.70 ± 2.65 MPs/g	17	10 % KOH	LDIR	(Zhu et al., 2023)
	> 1 µm PE > PVC > PA	126.8 ±147.5 mg/g	62	10 % KOH	FTIR, Py-GC/MS	(Garcia et al., 2024)
	1.03 – 6.84 µm PTFE, PS, ABS (48 %)	40 MPs in 31 samples	50	15 % KOH	µ-Raman	(Yun et al., 2024)
Testis	20 – 100 µm PS > PVC, PE > PP (100 %)	11.60 ± 15.52 MPs/g	6	10 % KOH	LDIR	(Zhao et al., 2023)
Skeletal tissue	11 to 151 µm PET > PE > rayon > PES (72 %)	5.24 ± 2.07 MPs/g	45 hip and knee synovium	30 % H_2O_2	µ-FTIR, µ-Raman	(Li et al., 2024)
	159.5 ± 103.8 µm PP > EVA > PS (~ 90 %)	61.1 ± 44.2 MPs/g	8 Intervertebral discs	Fenton´s reagent	µ-Raman	(Yang et al., 2025)
	138.86 ± 105.67 µm PP > EVA > PS (~75 %)	22.9 ± 15.7 MPs/g	8 bones			
	87.5 ± 30.7 µm EVA > PP > PS (~ 90 %)	26.4 ± 17.6 MPs/g	8 cartilages			

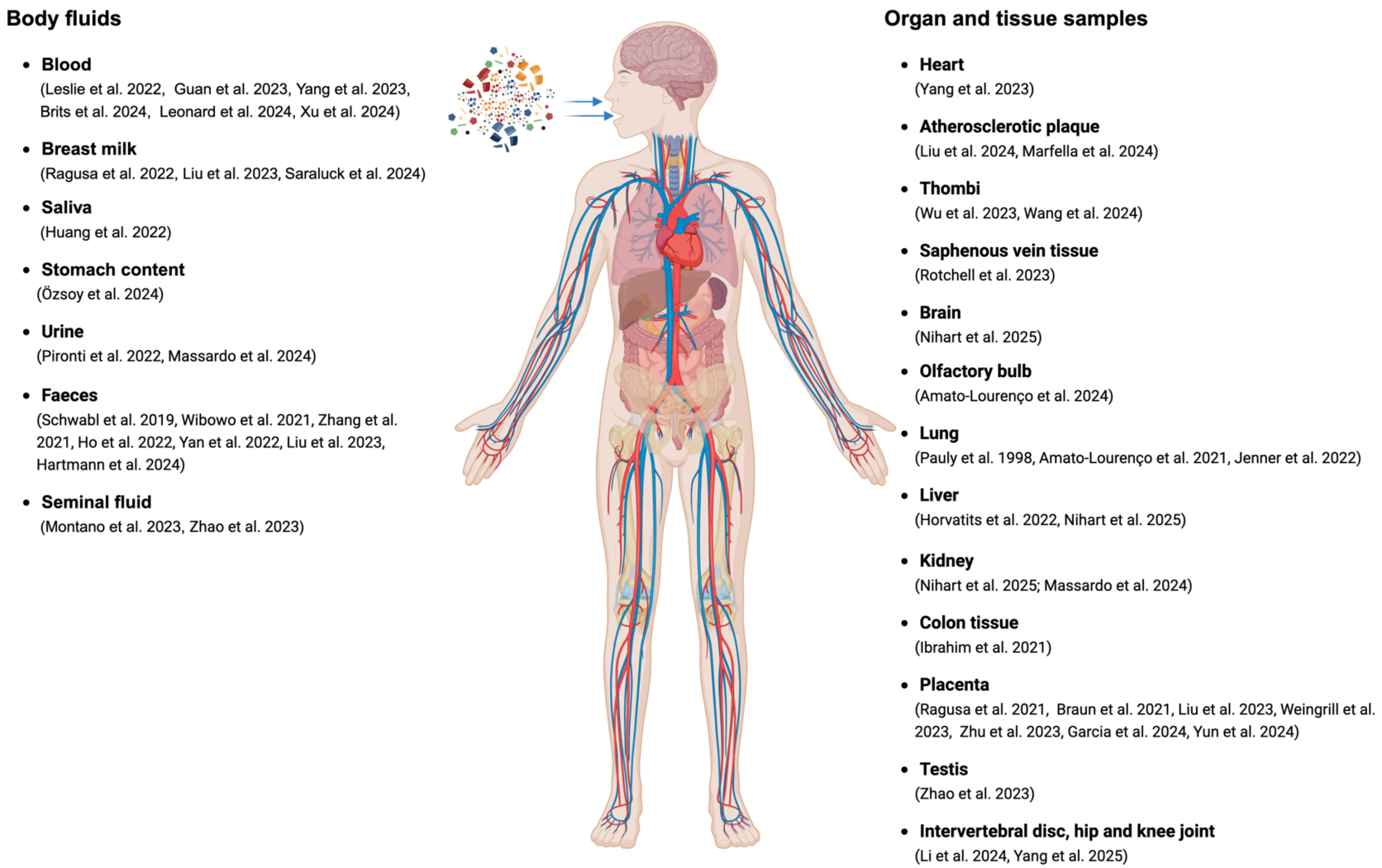

Figure 3. Ingestion and inhalation of plastic particles and their evidence to date in the human body. Based on the available literature, it can be inferred that plastic particles have been detected throughout the human body, including various organs, tissues, biological fluids, bones and cartilage (Created in BioRender. Geppner, L. (2025) https://BioRender.com/q69s186).

1.4 Research questions and objectives

In the course of this work, three studies were brought together on the basis of their common focus: (1) the development of an enzymatic method for the isolation of plastic particles from human blood, (2) the methodological comparison of different digestion protocols for particle extraction from mammalian tissue samples, and (3) a systematic review of potential impact of circulating plastic particles on blood pressure in humans.

The initial research focus concentrated on the methodological challenge of isolating plastic particles from human blood in a manner that is non-destructive, efficient, and ecologically sustainable. In order to address this challenge, a digestion protocol was developed using pepsin and pancreatin, which reflect the physiological digestion conditions of the human gastrointestinal tract. Therefore, a comparative analysis is necessary to provide a clear answer. This analysis will determine whether pepsin and pancreatin, which are used as less aggressive chemical digestion methods that preserve the integrity of plastic particles, can be used to make promising blood-based plastic analyses.

Building on these results, the second study investigated whether enzymatic digestion could be extended to mammalian tissue samples. However, due to the higher structural complexity of tissues, neither pepsin and pancreatin nor proteinase K achieved complete digestion, highlighting the limitations of enzyme-based methods in this context. Given the time and labor-intensiveness of enzymatic protocols, an investigation was conducted into the potential of potassium hydroxide as a chemical alternative. This investigation aimed to address the question of whether these conditions could effectively remove the organic matrix without altering the integrity of the particles.

The third publication focuses on the more general effects of systemic plastic exposure from a toxicological perspective. Although standardized detection methods are still lacking, numerous studies have demonstrated the presence of plastic particles in the human body. This raises the question of whether circulating plastic particles may contribute to changes in blood pressure.

In summary, the overarching research objectives of this thesis are:

(1) Establishment of a novel digestion method using pepsin and pancreatin to isolate plastic particles from human blood.

(2) Evaluation of pepsin and pancreatin for the isolation of plastic particles from mammalian tissue and establishment of a plastic-preserving alkaline digestion method.

(3) Investigation of the effects on human blood pressure caused by ingested and inhaled plastic particles that enter the bloodstream.

2 Research studies

2.1 A novel enzymatic method for isolation of plastic particles from human blood

Geppner, L., Ramer, G., Tomasetig, D., Grundhöfer, L., Küss, J., Kaup, M., & Henjakovic, M. (2023). A novel enzymatic method for isolation of plastic particles from human blood. *Environmental Toxicology and Pharmacology*, *104*, 104318. https://doi.org/10.1016/j.etap.2023.104318

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2.1.1 Contribution

CRediT authorship contribution statement

Liesa Geppner: Methodology, Formal analysis, Investigation, Validation, Writing – original draft. Georg Ramer: Formal analysis, Investigation, Writing – review & editing. Daniela Tomasetig: Formal analysis, Investigation. Leon Grundhöfer: Formal analysis, Investigation. Julien Küss: Formal analysis, Investigation. Marvin Kaup: Formal analysis, Investigation. Maja Henjakovic: Conceptualization, Methodology, Formal analysis, Investigation, Supervision, Project administration, Writing – original draft, Writing – review & editing.

Indication of own contribution

For the following publication, my primary contributions included conducting the laboratory research and analysing the data and images. I independently carried out all experimental investigations and processed the resulting data to ensure the validity and accuracy of the results. My role also involved collaborating with our cooperation partners Georg Ramer and

Daniela Tomasetig in the preparation and visualization of the samples, ensuring accurate representation of the microplastic structures and Raman spectra in the Raman microspectroscopic investigation. Together with my supervisor Maja Henjakovic, I contributed to the statistical analysis to support the conclusions of the study and co-authored the original manuscript. Additionally, my responsibilities included assisting in processing the editor's and reviewer's comments during the revision and resubmission process. Furthermore, I conducted a comprehensive literature review to provide context for our findings and support a robust scientific discussion.

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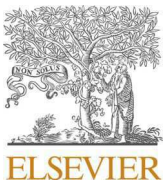

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A novel enzymatic method for isolation of plastic particles from human blood

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ABSTRACT

Microplastic particles have been detected in the human body.

This study aimed to develop a blood digestion method that preserves microplastics during analysis.

Acidic and alkaline reagents, commonly used for isolating plastic particles from organic materials, were tested on human blood samples and microplastics. Nitric acid, hydrochloric acid, potassium hydroxide, and sodium hydroxide were examined over time. Additionally, a pepsin-pancreatin combination was utilized for blood digestion. Light microscopy assessed digestion efficiency and particle count changes, while Raman micro-spectroscopy distinguished between plastic and cell debris.

The acidic reagents were ineffective in removing the organic material, while alkaline reagents were effective without significant effects on microplastics. Blood digestion using pepsin and pancreatin demonstrated efficient digestion without negative consequences for the particles.

While potassium hydroxide digestion is already established, novel use of the pepsin-pancreatin combination was introduced to digest human blood, indicating its potential for isolating plastic particles from tissue and human food.

1. Introduction

Potential health risks due to the accumulation of microplastic particles in the human body have already been described and discussed in many different contexts (Campanale et al., 2020; Kutralam-Muniasamy et al., 2022).

Environmental scientists have established numerous methods to isolate plastic from organic material (e.g. sewage sludge, sediment, water). They have mainly used acidic and alkaline methods, but some enzymatic methods were also adapted (Kühn et al., 2017; Li et al., 2020; Pfeiffer and Fischer, 2020). It should be noted, however, that in some cases completely different conditions prevail in the organic environmental material than in the blood. Additionally, larger plastic particles play a role, while the focus of the blood examination is primarily on very small microplastic and nanoplastic particles (Al-Azzawi et al., 2020).

There is certainly a need to find methods for the detection of micro- and nanoplastics in human samples. After all, microplastics have already been detected in human body fluids and tissues. The first enzymatic method to detect the presence of plastic particles in human blood was described by Leslie and colleagues (Leslie et al., 2022). Senathirajah and colleagues calculated that on average humans can absorb 0.1–5 g of microplastics per week (Senathirajah et al., 2021). In further studies microplastic has been found in human lung tissues (Amato-Lourenço et al., 2021), in human placental tissue (Ragusa et al., 2021) and even migrates through the gastrointestinal tract which provides evidence of microplastic detection in the stool (Schwabl et al., 2019).

As many possibilities exist for dissolving the organic matrix as there are for the subsequent identification and quantification of the plastic particles (Ivleva, 2021). In the course of this study, an attempt was made to develop a simple, non-particle-destructive, time-saving, low-cost and easily accessible method of particle isolation from human blood. Moreover, a careful and cost-effective method of blood digestion using the enzymes pepsin and pancreatin was successfully described for the first time.

In accordance with the results of Leslie and co-workers (Leslie et al., 2022), and due to the fact that polystyrene (PS) particles have been

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widely used in animal microplastic studies (Schwarzfischer et al., 2022; Shan et al., 2022; Vlacil et al., 2021), as well as the fact that this type of plastic particle is very easy, commercially and cheaply available, 5 μm polystyrene (PS) plastic particles were used for the research purposes of this study.

2. Material and method

2.1. Sample collection

The whole blood used for this investigation was obtained from anonymized volunteers, who signed a patient information and informed consent in accordance with the regulations of the Ethics Committee in force in Austria. This study was approved by the Academic Integrity and Ethics Committee of the Danube Private University (DPU-EK/010).

The samples were collected in 10 mL EDTA vacutainers (367525, BD Biosiences, Vienna, Austria) or in 6 mL glass heparinized vacutainers (268886, BD Biosiences, Vienna, Austria). Venipuncture was done by sterile Vacutainer-Safety-Lok 21 G (367282, BD Biosiences, Vienna, Austria). Immediately after sampling, the blood samples were stored in a –20 °C freezer until analysis.

2.2. Microscopy

A light microscope (Leica DM2500 LED, Leica Microsystems AG, Balgach, Switzerland) was used for visualization of alkaline and enzyme-dependent digestion and counting of plastic particles. Unless otherwise stated, the pictures were taken with 40-fold magnification.

Raman spectra were obtained using a confocal Raman microscope (alpha 300RSA+, WITec Wissenschaftliche Instrumente und Technologie GmbH, Ulm, Germany) to investigate effects of alkaline and enzymatic reagents on plastic particles and determine their compositions. For efficient imaging at spatial resolution in the low micrometer range, spectra were recorded with 100x magnification (Zeiss objective EC Epiplan-Neofluar Dic, N.A. 0.9). The 532 nm diode laser with a laser power adjusted to 10 mW was used for Raman excitation. A spectral resolution of approximately 1 cm^{-1} was achieved employing a 600 groove/mm grating (Wieland et al., 2017). All measurements were performed under ambient conditions and at moderate laser power, to avoid heating effects.

2.3. Digestion

2.3.1. Acid digestion method

For acid digestion, blood samples were treated with 1 M hydrochloric acid (HCl, 6792.1, Carl Roth GmbH + Co. KG, Karlsruhe, Germany) or 1 M nitric acid (HNO_3, 9887.2, Carl Roth GmbH + Co. KG, Karlsruhe, Germany). The used ratios of acid solution to sample were 6:1, 3:1, and 1.5:1. First, each acid volume was transferred to an autoclaved 100 mL Erlenmeyer flasks (Duran®, Carl Roth GmbH + Co. KG, Karlsruhe, Germany), the EDTA or heparinized blood samples were added. The flasks were incubated in a water bath at 45 °C for 24 h. Changes in human blood samples was checked using light microscopy at the respective time points: 0 h (immediately after preparing the mixture), 0.25 h, 0.5 h, 1.5, 4 h and 24 h.

2.3.2. Alkaline digestion method

For alkaline digestion, blood samples were treated with 1.8 M (10 %) potassium hydroxide solution (KOH, P747.1, Carl Roth GmbH + Co. KG, Karlsruhe, Germany) or 1 M sodium hydroxide solution (NaOH, 6785.1, Carl Roth GmbH + Co. KG, Karlsruhe, Germany). The used ratio of alkaline reagent to sample was 4:1. First, KOH and NaOH solutions were transferred to an autoclaved 100 mL Erlenmeyer flasks, then EDTA blood samples were added. The flasks were incubated in a water bath at 45 °C for 24 h. Digestion progress was checked using light microscopy at the respective time points: 0 h (immediately after preparing the mixture), 0.25 h, 0.5 h, 1.5, 4 h and 24 h. The effect of alkaline reagents on blood samples was investigated in minimal twenty pictures across two cover slips per sample and the amount of cell debris was determined using Fiji (ImageJ, version 2.1.0/1.53c, National Institutes of Health).

2.3.3. Enzymatic digestion method

Porcine pepsin (KK38.1, Carl Roth GmbH + Co. KG, Karlsruhe, Germany) has a specific enzyme activity of ≥ 2000 FIP-U/g as provided by the manufacturer. For the stock solution, pepsin was dissolved in 10 mM HCl to obtain a concentration of 1 mg/mL.

Porcine pancreatin (A0585, AppliChem GmbH, Darmstadt, Germany) has a specific enzyme activity of 36000 FIP-U/g as provided by the manufacturer. For the stock solution, pancreatin was dissolved in Dulbecco's phosphate-buffered saline (DPBS, 14190-144, Thermo Fisher Scientific, Vienna, Austria) to obtain a concentration of 1 mg/mL.

First step of enzymatic digestion protocol was the denaturation and pre-digestion of blood by HCl and pepsin. For optimal activity of pepsin, heparinized blood with added HCl solution (160 mM in end volume) were mixed in a 100 mL Erlenmeyer flasks and were pre-incubated in a water bath for 5 min at 37 °C before 1 mg pepsin per mL blood was added.

After incubating the blood samples in the pepsin solution at 37 °C for 4 h, the mixture was buffered to a pH between 7 and 7.4 by adding of 1 M sodium hydrogen carbonate ($NaHCO_3$, 8551.1, Carl Roth GmbH + Co. KG, Karlsruhe, Germany). After neutralization, 2 mg pancreatin per mL blood was added. Subsequently, 2 mg of pancreatin per mL blood was added four more times within 24 h. Enzymatic digestion was investigated using light microscopy after 4 h incubation time of pepsin, 0.5 h (after first addition of pancreatin), 20 h (after third addition of pancreatin) and 24.5 h (30 min after the fifth and last addition).

Furthermore, an investigation was made to see if it is possible to simplify the enzyme protocol by adding pancreatin only once, without making major losses in the digestion progress. Except for pancreatin, all quantities, concentrations and incubation times were maintained as previously described. After neutralization, 10 mg pancreatin per mL blood was added as single dose and incubated for 24.5 h. Enzymatic digestion was investigated using light microscopy, this time only after 4 h incubation time of pepsin and 24.5 h incubation time of pancreatin.

The effect of enzymes on blood samples were investigated in minimal twenty pictures across two cover slips per sample and the amount of cell debris was determined using Fiji (ImageJ, version 2.1.0/1.53c, National Institutes of Health).

2.4. Effect of digestion protocols on plastic particles

We have tested polystyrene (PS) particles to investigate their change of different digestion solutions. For this purpose, polystyrene polymer particles with a size of 5 μm were purchased from BS-Partikel GmbH (HS0500–20, Mainz, Germany). These utilized commercially available microplastic particles, were produced in a standardized manner and characterized by the manufacturer. The mean diameter of these 5 μm particles is 4.96 μm with a coefficient of variation of 1.6 %. They are made of poly(styrene-co-divinylbenzene), have a density of 1.05 g/cm3, and a refractive index of 1.59 at 25 °C.

The effect of the different digestion reagents on polystyrene (PS) particles were tested. For this purpose, 40 μg/mL of 5 μm particles were added to alkaline or enzymatic digestion solution.

For the alkaline controls, blood and blood with alkaline reagent were replaced by particle free H_2O (A511.2, Carl Roth GmbH + Co. KG, Karlsruhe, Germany). Respective time points for alkaline solution were shortened to: 0 h (immediately after preparing the mixture), 0.5 h, 1.5 h, 4 h and 24 h for the 5 μm particles at 45 °C. Similarly, the particles were applied in enzymatic digestion, all at 37 °C. In this case the respective time points are 4 h after pepsin insertion and 0.5 h, 24.5 h, 48.5 h and 72.5 h after the first addition of pancreatin.

Effects of these reagents on the number of PS particles were checked

using conventional light microscopy (Leica DM2500 LED, Leica Microsystems AG, Balgach, Switzerland). The changes in the number of particles were investigated in minimal twenty pictures across two cover slips per sample and were analyzed using Fiji. Additionally, effects in the size as well as changes in the surface structure at 5 μm isolated particles from alkaline and enzymatic digestion solution were investigated using Raman microscopy (alpha 300RSA+, WITec Wissenschaftliche Instrumente und Technologie GmbH, Ulm, Germany).

2.5. Statistical analysis

All data are represented as means ± SD. A minimum of three independent experiments were performed to establish each single data point. Statistical analyses were performed using the two-tailed unpaired t-test when testing between two time points and one-way analysis of variance with Dunnett's multiple comparison test was performed for within-group comparisons (GraphPad Prism9, version 9.3.1). Statistical significance was set at $P < 0.05$.

3. Results

3.1. Inefficient acid digestion

Previous reports have identified oxidizing and reducing acids as possible digestive solutions for organic materials (e.g., mussels, fish, or marine sediment). Therefore, we tested 1 M HNO_3 and 1 M HCl with regards to blood digestion efficiency in 100 mL Erlenmeyer flasks for 24 h at 45 °C. Surprisingly, blood coagulation was induced by addition of HNO_3 or HCL (Fig. 1A). Higher acid volumes, longer incubation time, EDTA and heparinized blood samples were tested, but no digestive effects were observed (Fig. 1B).

3.2. Efficient alkaline digestion with alteration of particles

In addition, alkaline digestion of blood samples was tested. Therefore, blood samples were treated with a 1.8 M KOH (10 %) or a 1 M NaOH and amount of cell debris was analyzed using light microscopy. Fig. 2 shows that a considerable progress in digestion can already be seen after 24 h. Only small, isolated, undigested cell debris remains after 24 h compared to the starting point of digestion (0 h).

The graphic representation in Fig. 3 shows that digestion already progresses significantly in the first 15 min. Whereas the area of undigested cell debris can be assumed to be 100 ± 28.9 % at the starting point (0 h), it already decreases to 24 ± 4.5 % after 15 min for KOH, respectively from 100 ± 41.4 % (0 h) to a reduction of 28 ± 7.0 % after 15 min for NaOH. After 24 h, the area of undigested cell debris has decreased to only 12 ± 2.5 % and 12 ± 2.2 % for KOH and NaOH, respectively.

Based on the fact that according to the literature (Al-Azzawi et al., 2020) KOH alters particles in some cases, this was tested on polystyrene microplastic particles. For this purpose, the 5 μm PS plastic particles were counted in different solutions at the respective time points. Fig. 4A shows the percentage amount of plastic particles counted in 1.8 M KOH (10 %) solution with presence and absence of human blood and in a H_2O control attempt, after 0, 0.5, 1.5, 4 and 24 h incubation time.

Slight fluctuations in the number of particles at the different incubation times can be seen. A statistically significant decrease in the number of particles after 24 h of incubation at 45 °C was not observed. Similar results were obtained with NaOH (Fig. 4B). No statistically significant change in the number of polystyrene particles incubated in NaOH was observed in this case either.

Analysis using confocal light microscopy shows that the surface structure of 5 μm PS particles is not altered by alkaline solutions (Fig. 5A). Slight irregularities around the surface are only visible in the PS particles that were incubated in blood and alkaline solution. To make sure these are indeed cell debris and not decreased and / or altered particles, Raman spectra were acquired and analyzed. Comparing the spectra of the unknown structures with spectra made from indeed PS particles, the significant spectral differences show that the unknown structures do not belong to PS particles (Fig. 5B).

3.3. Efficient enzymatic digestion without damage of particles

As a further method of blood digestion, pepsin (Pep) and pancreatin (Pan) were used. After incubation with pepsin, pancreatin was added stepwise and microscopic images were taken at specific time intervals. At *0.5 h Pan*, microscopy was performed after single addition of pancreatin, at *20 h Pan*, it was performed after triple addition of pancreatin and at *24.5 h Pan*, microscopy was performed after the entire fivefold amount of pancreatin was added.

Large areas of cell debris and blood cells are still visible after 4 h of pepsin addition (4 h Pep), but with increased pancreatin intake, digestion progresses significantly, and blood is almost completely digested after 24.5 h (Fig. 6).

This effect can also be observed in the statistical evaluation in Fig. 7A. To investigate whether a comparable improvement in digestion could also be achieved with a simplified protocol, which involved adding the entire amount of pancreatin once, the protocol was adapted and performed accordingly. Fig. 7 shows that both digestion protocols achieve similarly good results. In both graphs, when comparing the initial value of pepsin after 4 h with the final value of pepsin and pancreatin after 24.5 h, the area of cell debris decreases to less than 5 % (decrease in cell debris between the first and the last measurement is 98.1 % in Fig. 7A and 95.8 % in Fig. 7B).

In addition, an investigation was made to see whether these enzymatic digestion conditions alter PS particles. The difference in the numbers of 5 μm PS particles is minimal and significant difference is only noticeable after 24.5 h. However, after 72.5 h and at all other

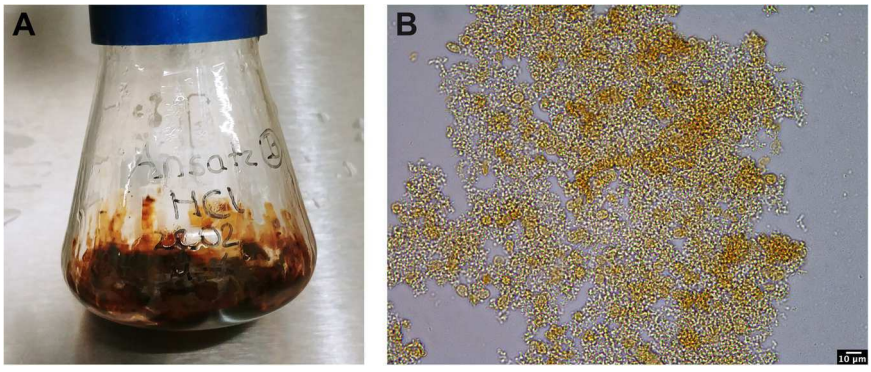

Fig. 1. Acid digestion of blood samples using 1 M HCl. (A) The image depicts coagulated and clotted blood at 0 h incubation time in HCl solution, as microscopic examination was not feasible. (B) Representative microscopic image shows high amount of digestive remains after 24 h incubation at 45 °C.

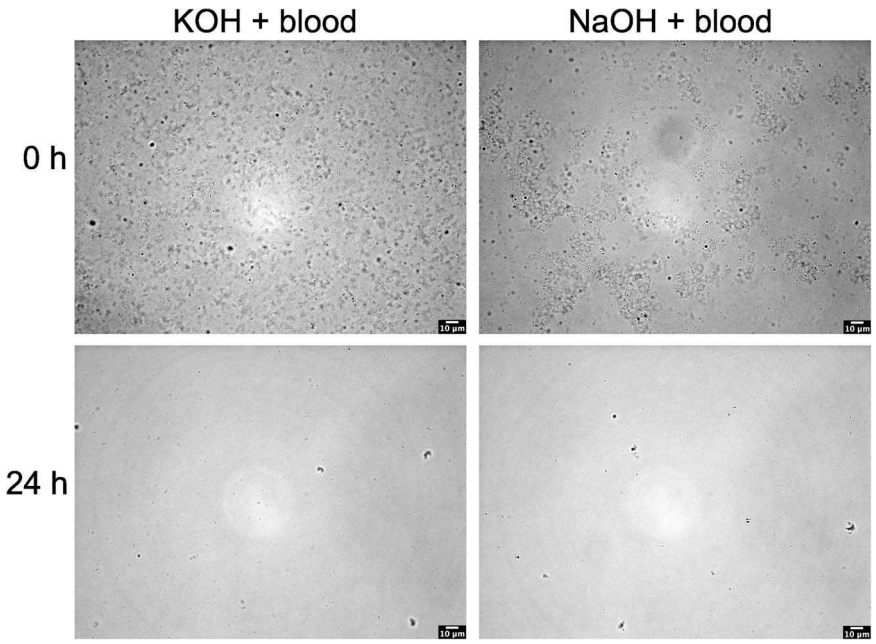

Fig. 2. Alkaline digestion of blood samples using 1.8 M KOH or 1 M NaOH at an incubation temperature of 45 °C. Representative microscopic images show digestion progress between 0 h and 24 h of incubation at 45 °C.

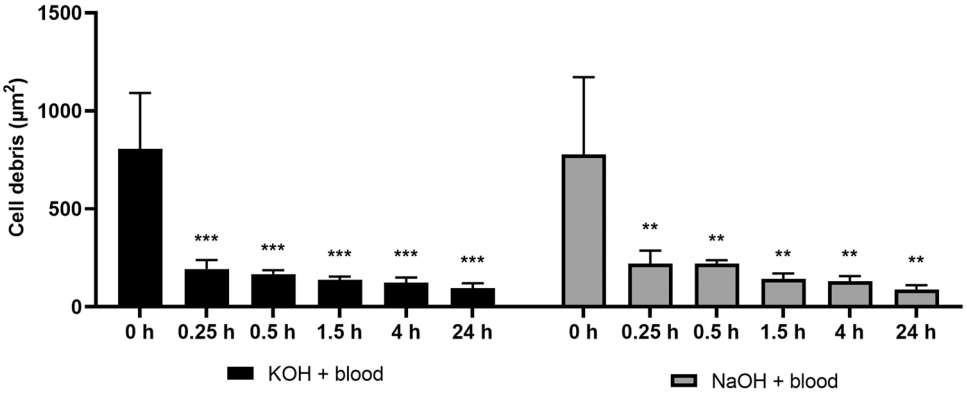

Fig. 3. Time-dependent alkaline digestion of blood samples using 1.8 M KOH or 1 M NaOH at an incubation temperature of 45 °C. Cell debris were detected by light microscopy and subsequent image analysis with a Fiji macro where only cell debris were selected with the help of the threshold tool and then their area was measured. Data are presented as mean ± SD. $n_{(KOH+blood)} = 3$, $n_{(NaOH+blood)} = 3$. Statistical significance was determined by using a one-way analysis of variance with Dunnett's multiple comparison test for within-group comparisons. * *$p < 0.01$ and ***$p < 0.001$, within the group significantly different from 0 h.

investigated time points, no significant differences were found (Fig. 8A). Furthermore, a confocal light microscopic examination could not detect remarkable changes in the surface texture of the 5 µm PS particles (Fig. 8B). The difference between a Raman spectrum of a 5 µm PS particle and an undefined organic debris can be seen in Fig. 8C.

4. Discussion

The aim of this study was to establish a simple and economical method by which blood could be digested successfully and at the same time the PS particles would not be damaged. For this purpose, five digestion reagents, some of which are frequently used (HNO_3, HCl, KOH and NaOH), and a novel one (pepsin / pancreatin) was applied for nondestructive isolation of PS plastic particles.

Various methods have already been compared in the literature that can be used for a harmonized and cost-effective analysis of microplastics (Primpke et al., 2020). According to them, the most cost-affordable technique for the rapid identification of PS plastic particles in blood, is the use of light microscopy. Another benefit of this technique is that most laboratories are equipped with an appropriate microscope.

Raman microscopy is another method that has been used very frequently throughout the literature, especially in relation to the analysis of microplastics (Ivleva, 2021). Even though Raman microscopy has long measuring and working times, and can be expensive, it seems to be the most appropriate method to investigate microplastics <10 µm (Primpke et al., 2020). This is mainly due to the combination of the method with clear chemical particle identification and morphological characterization.

For this reason, our study selected these two methods, using light microscopy as the primary method of investigation, and Raman microscopy and spectroscopy as a chemical and morphological verification.

One of the aspects another study compared was acid-based digestion and its influence on microplastics (Al-Azzawi et al., 2020). Good acid-induced digestion effects were achieved in the digestion of organic material such as fish and mussels. In the present study, independent on the incubation time and / or acid concentration blood was not digested by HNO_3 and HCl.

No digestive effect of acids on blood samples suggests that calcium

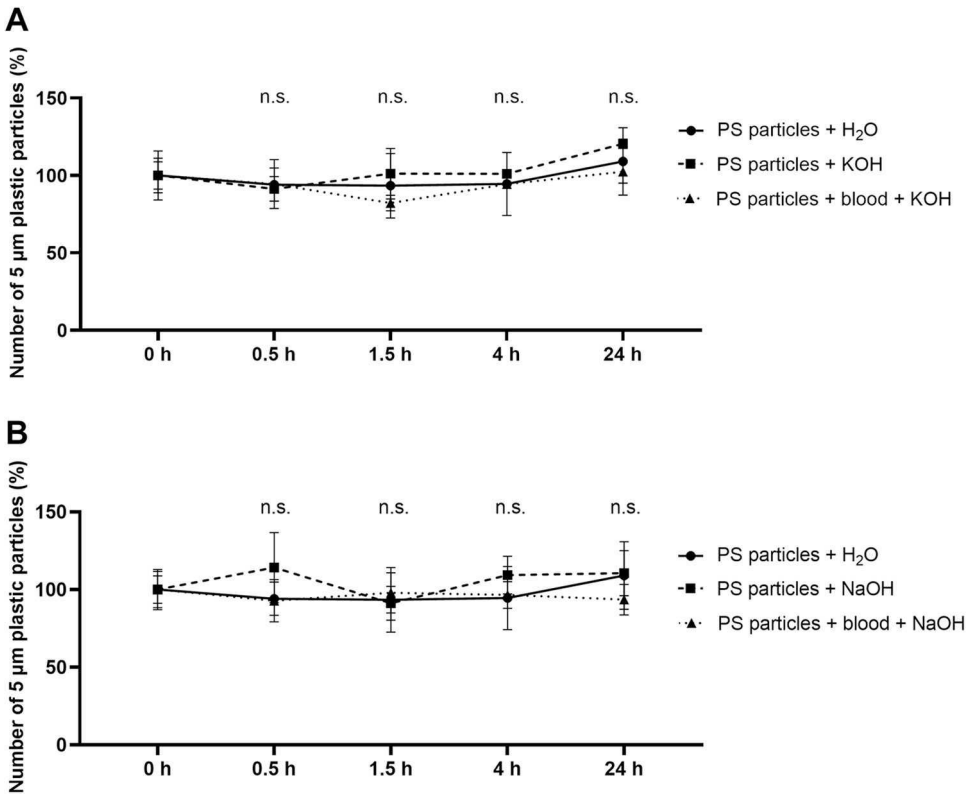

Fig. 4. Effect of (A) 1.8 M KOH and (B) 1 M NaOH on 5 µm polystyrene (PS) plastic particles. PS particles were incubated with 1.8 M KOH or 1 M NaOH in presence and absence of human blood and in H_2O control at 45 °C for up to 24 h. PS particles were counted using the Fiji cell counter tool. Data represents mean ± SD. n = 6 for H_2O control and n = 3 for KOH and NaOH in presence and absence of human blood. Statistical significance was determined by using a one-way analysis of variance with Dunnett's multiple comparison test for within-group comparisons to the results at 0 h which showed no statistical significance (n.s.).

ions in EDTA complex were displaced by protons, which induced blood coagulation. Another reason for coagulation of heparinized blood samples in presence of HNO_3 or HCL could be acid-dependent suppression of antithrombin.

It turned out that at high molarity of HNO_3 (22.5 M) and high temperatures, which include short boiling (30 min), there is a large loss of polystyrene microplastics (Gulizia et al., 2022). At lower molarities and lower incubation temperatures, no effects were found on the PS microplastics (Gulizia et al., 2022). Another study describes the destructive effects of HNO_3 and HCl on polyethylene (PE, low-density and high-density), polypropylene (PP) and polyethylene terephthalate (PET) (Karami et al., 2017) with recovery rates below 95 %, which should be considered when determining other polymers.

Therefore, we chose not to pursue the optimization of ineffective blood disruption using HCl and HNO_3 because a prior study demonstrated the existence of plastic particles consisting of different types of plastic, such as PE, PP, and PET, in the bloodstream (Leslie et al., 2022). Additionally, since this investigation only employed polystyrene model particles, we would be unable to evaluate the destructive influence of these reagents.

A very simple, effective and widely used method of digesting a wide variety of organic materials (water samples, mice organs, human placenta), is the method of digesting with KOH (Alfonso et al., 2021; Ragusa et al., 2021; Schwarzfischer et al., 2022). Ragusa and colleagues used this method to dissolve the human placenta and were able to extract microplastics between 5 and 10 µm in size (Ragusa et al., 2021). For the KOH digestion method, there are different results regarding its influence on plastic particles. KOH digestion is described as nondestructive for PS microplastics even under the most extreme conditions (90 °C and 48 h) (Gulizia et al., 2022), while Thiele and colleagues recommend a digestion temperature of 40 °C, which leads to a reasonable recovery rate of microplastics (Thiele et al., 2019). The study by Alfonso and colleagues comes to similar conclusions. While PET shows size changes in KOH digestion at 60 °C and 72 h, no size changes could be detected in all tested polymers at 40 °C and 72 h incubation time (Alfonso et al., 2021). But for some reason there seems to be degradation of PS particles, even with very mild KOH treatment, especially in combination of KOH and H_2O_2 (López-Rosales et al., 2021).

To investigate the influence of solutions on microplastics in this study, polystyrene plastic (PS) particles were used, which are well and cheaply available and have also been successfully used *in vivo* and *in vitro* experiments (Al-Azzawi et al., 2020; Sarma et al., 2022; Shan et al., 2022).

No significant alterations in frequency or shape were observed for the 5 µm particles incubated in KOH in this study. Only slight fluctuations in the frequency of the PS plastic particles are recognizable, which are due to the tendency of the PS particles to adhere to larger clusters. In H_2O, however, the particles are isolated and do not form larger particle groups. Surface irregularities in particles incubated in blood and alkali can be attributed to the cell debris that adhere to the particles.

Due to the fact that Cole and colleagues additionally tested the digestion efficiency of 1 M NaOH and achieved good results, NaOH digestion has also been tested in the present study (Cole et al., 2014). It shows that blood can already be dissolved well even at mild temperatures (37 °C).

Depending on the concentration, NaOH has been shown to have a degrading effect on polycarbonate (PC) and PET (Hurley et al., 2018) and to cause damage and discoloration in polyethylene (PE), Nylon and unplasticized polyvinylchloride (uPVC) (Cole et al., 2014). The insight that there are no degrading effects on PS plastic was also the conclusion of the present study, as no changes were observed in 5 µm PS particles incubated in NaOH.

Overall, no changes caused by KOH and NaOH can be observed with 5 µm PS particles. All the studies mentioned above, which did not report

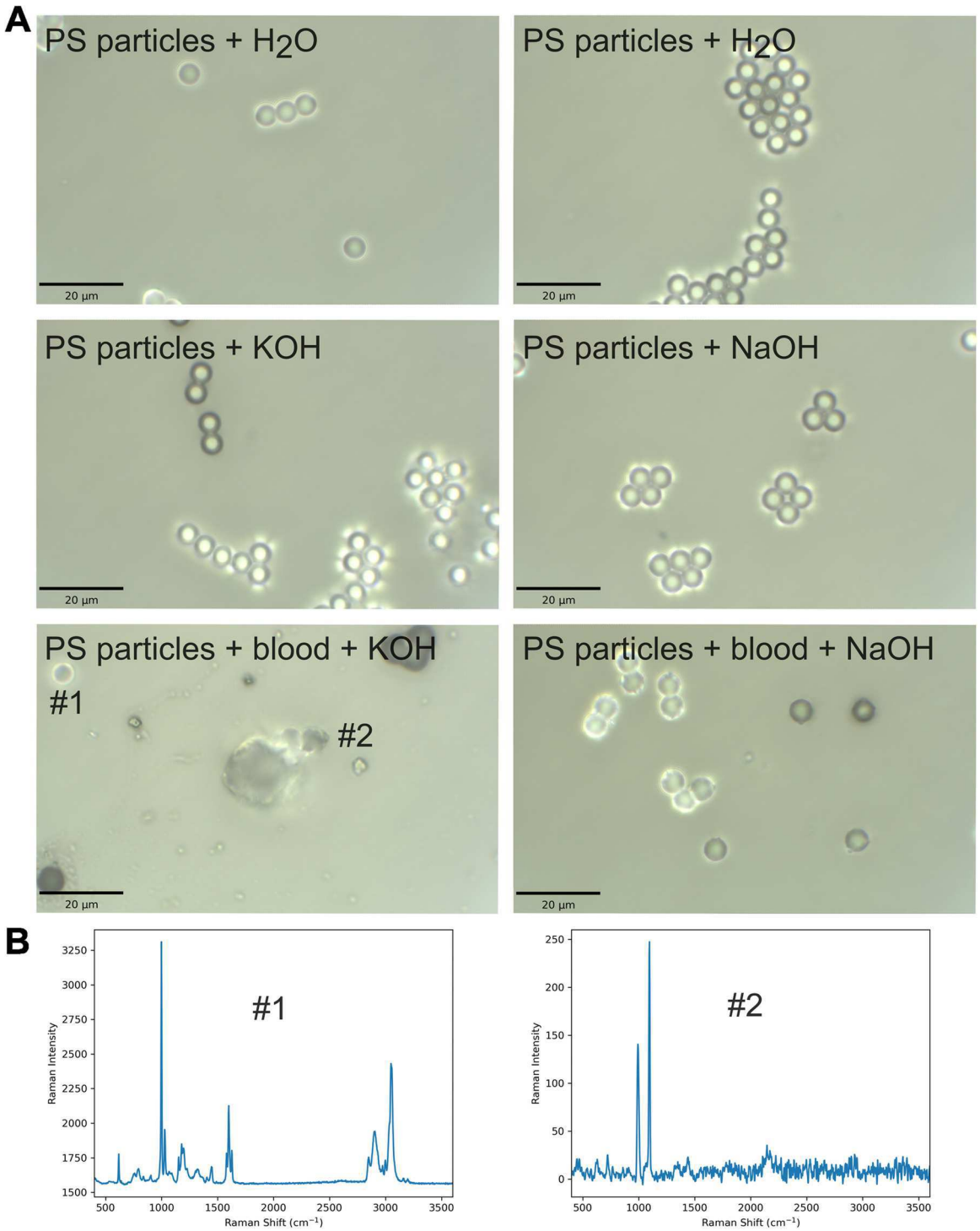

Fig. 5. Raman microspectroscopic investigation of 5 µm PS particles after 24 h incubation in H_2O, KOH and NaOH at 45 °C. (A) Particles were visualized using confocal light microscopy. (B #1) representative Raman spectrum of a 5 µm PS particle; (B #2) representative Raman spectrum of an organic remnant.

any changes in PS particles, used larger microplastic particles (<200 µm). Due to the lower surface to volume ratio, the particles are potentially less susceptible to the digestive reagents. Further studies need to be undertaken in order to be able to make a reliable statement that KOH does not make any changes at 45 °C, even for smaller microplastic or nanoplastic particles.

Furthermore, another method for blood digestion was used that is characterized by its biological specificity and milder properties – enzymatic digestion. In a recent study, microplastics were detected in the blood for the first time by using proteinase K as an enzymatic digestion process (Leslie et al., 2022). Accordingly, we have optimized a more economic and simplified digestion protocol with pepsin and pancreatin enzymes capable of dissolving and digesting blood without causing damage to microplastics.

The proteinase K digestion method requires more working steps and can be more expensive due to the additional cost of the enzyme, but the pepsin and pancreatin method is similar in terms of the effort required, although with slightly fewer steps. The digestion period for pepsin and pancreatin is a little longer, but it is a more cost-effective option. Even if the matrix is not completely dissolved, the spectroscopic analysis is not

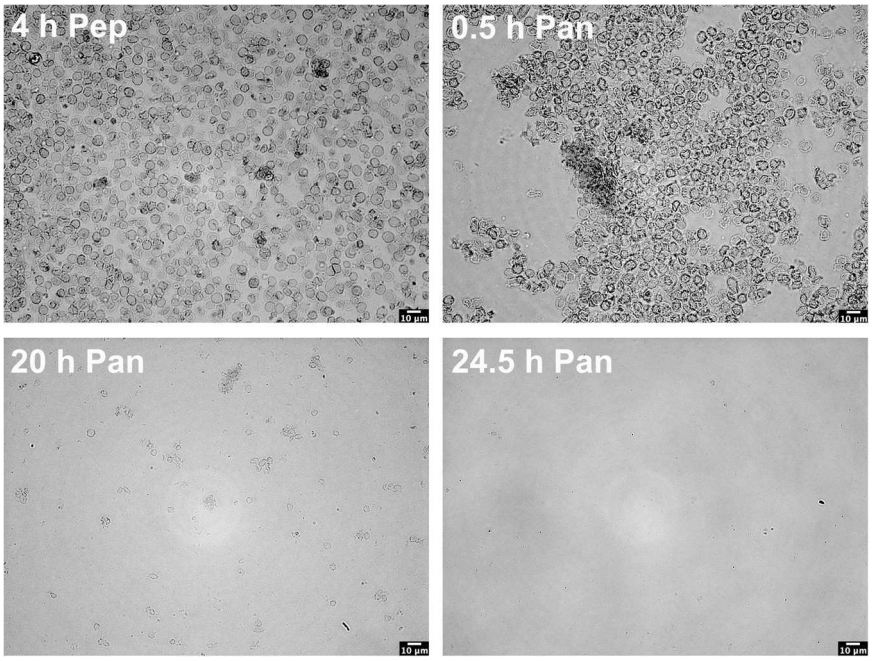

Fig. 6. Enzymatic digestion of blood samples using pepsine (Pep) and pancreatine (Pan). Representative microscopic images show digestion progress between 4 h incubation with only pepsine and 24.5 h incubation with pancreatin, at 37 °C.

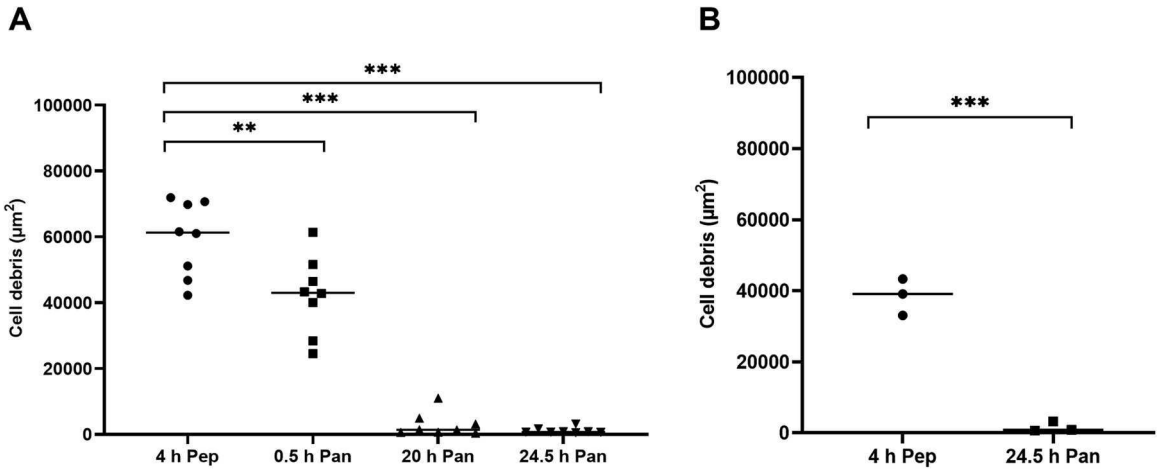

Fig. 7. Enzymatic digestion of blood samples using pepsine (Pep) and pancreatine (Pan). (A) Digestive progress with gradual addition of pancreatin (n = 8). (B) Digestive progress with one complete addition of pancreatin (n = 3). Cell debris were detected by light microscopy and subsequent image analysis with a Fiji macro where only cell debris were selected with the help of the threshold tool and then their area was measured. Data points represent experimental repetition and crossbar represents median. Statistical significance was determined by using a one-way analysis of variance with Dunnett's multiple comparison test for within-group comparisons (A) and a two-tailed unpaired t-test (B). **$p < 0.01$ and ***$p < 0.001$, significantly different from 4 h Pep.

restricted and the plastic particles can still be analyzed well.

The enzymatic digestion protocol was adapted in a very simplified way to physiological digestion conditions. The physiological concentration of pepsin is at its optimum at a pH between 1.5 and 2.0 (Bardhan et al., 2012), which is why the pH was adjusted using HCl. The pepsin concentration in gastric juice is typically between 0.5 and 1 mg/mL, therefore a maximum pepsin concentration of 1 mg per mL blood was used in this study (Zhu et al., 2006). The optimal digestion efficiency of pancreatin was investigated at different pH and concentrations. The best digestion results were found to be at pH between 7 and 7.4 and a concentration of 10 mg per mL blood.

If the analytical methods for plastic particles are optimized, the isolation of these particles should be expedited, thus eliminating the need for a 24-hour handling period of samples in analytical laboratories. The use of higher enzyme concentrations can significantly accelerate the digestion of blood samples without incurring excessive analysis costs. The concentrations chosen in this study were deliberately low, with the pepsin concentration based on average physiological levels of the enzyme found in the stomach (Zhu et al., 2006). It is envisaged that we will attempt to further enhance the pepsin/pancreatin digestion process, making it faster and more efficient. This is because this enzymatic combination holds substantial potential for digesting a wide variety of samples, particularly when considering the gastrointestinal digestion in mixed nutrition.

After the digestion protocol was optimally adapted and good results of the digestion were achieved, it was further investigated whether the enzymes caused changes in PS particles. Both the particle counting and the Raman microscopic investigation of the particles showed that no

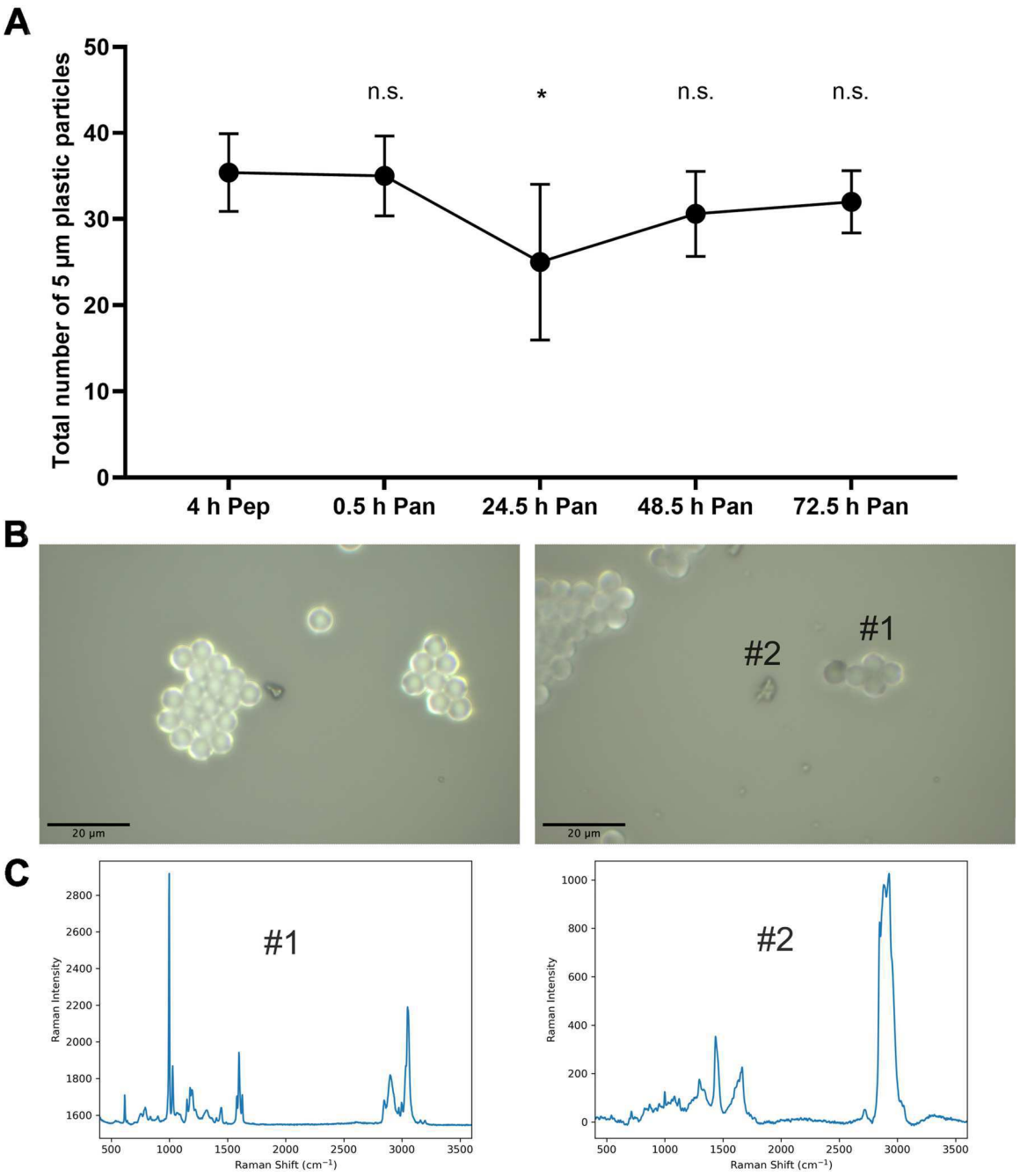

Fig. 8. Effect of enzymatic blood digestion using pepsin and pancreatin on 5 µm PS particles, incubated at 37 °C for 72.5 h. (A) Effect of digestive enzymes pepsin and pancreatin on total number of 5 µm PS particles. Data represents mean ± SD. n = 5. Statistical significance was determined by using a one-way analysis of variance with Dunnett's multiple comparison test for within-group comparisons. n.s.: not significant, *: $p < 0.05$: significantly different from 4 h Pep. (B) Particles visualized using a confocal light microscopy. Left: Accumulation of 5 µm particles; Right: #2 marks abnormality of which Raman spectra have confirmed that these are not altered PS particles. (C #1) representative Raman spectrum of a 5 µm PS particle; (C #2) representative Raman spectrum of an organic remnant.

significant numerical differences and surface changes were detectable with the 5 µm PS particles.

In summary, no effects of the alkaline and enzymatic digestion solutions used on 5 µm polystyrene microspheres have been detected after a thorough investigation. However, potential effects on other types of plastic cannot be completely ruled out. Regardless, the study chose this polystyrene model particle as it is commercially available and commonly used in microplastics research (Lee et al., 2022; Tsou et al., 2023). Moreover, these model particles correspond to the size of microplastics that may circulate in the bloodstream, and polystyrene is one of the most prevalent plastics found in the bloodstream (Leslie et al., 2022). An additional advantage of 5 µm PS model particles is that their size is detectable using selected analytical methods without reaching their limit of detection.

In the future, the analysis of microplastic particles will involve particles of various shapes and sizes and will encompass both individual plastic types and plastic mixtures within alkaline solutions. Enzymatic digestion with pepsin/pancreatin will also be used for analysis purposes. It is recommended to test the recovery of model particles of different plastic types when utilizing alkaline digestion with KOH and NaOH solutions. Assuming the conditions are appropriate, the integrity of microplastics can be assumed when utilizing enzymatic digestion with

pepsin/pancreatin.

Attempting to mimic the mixture of various plastic types as actually found in a sample, whether through grinding or laser ablation, is likely to be quite challenging. As long as such plastic particle mixtures are not standardized and commercially available, making comparisons of induced effects in different studies will remain unfeasible.

5. Conclusion

The initial intention of this study was to test through blood sample digestion methods and adapt and validate the most suitable method for microplastic detection. Therefore, different acidic, alkaline and enzymatic reagents were used. This led to the conclusion that, regardless of concentration and incubation time, acids did not sufficiently digest blood samples. Alkaline reagents showed good results regarding digestion without any changes of added 5 μm particles. However, in the case of alkaline solution such as KOH, further investigations should be carried out with smaller plastic particles and different plastic types. Additionally, another method was applied, using the enzymes pepsin and pancreatin as digestive agents. This study recommends both, the KOH and the enzymatic digestion with pepsin and pancreatin to be a harmless method for isolation and analysis of microplastic particles. Although more aggressive digestion methods appear to be applicable with larger microplastic particles.

Further work is required to trial the technique on field-collected blood samples and finally develop means for better visualization of microplastics or even nanoplastics that have entered the human bloodstream.

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CRediT authorship contribution statement

Liesa Geppner: Methodology, Formal analysis, Investigation, Validation, Writing – original draft. **Georg Ramer**: Formal analysis, Investigation, Writing – review & editing. **Daniela Tomasetig**: Formal analysis, Investigation. **Leon Grundhöfer**: Formal analysis, Investigation. **Julien Küss**: Formal analysis, Investigation. **Marvin Kaup**: Formal analysis, Investigation. **Maja Henjakovic**: Conceptualization, Methodology, Formal analysis, Investigation, Supervision, Project administration, Writing – original draft, Writing – review & editing.

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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2.2 Testing of Different Digestion Solutions on Tissue Samples and the Effects of Used Potassium Hydroxide Solution on Polystyrene Microspheres

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2.2.1 Contribution

Author contributions

L.G.: methodology, formal analysis, investigation, validation, writing—original draft. J.K.: formal analysis, investigation. W.W.: formal analysis, investigation. M.R.: formal analysis, investigation. T.G.: formal analysis, investigation. P.W.: formal analysis, investigation. M.H.: conceptualization, methodology, formal analysis, investigation, supervision, project administration, writing—original draft, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Indication of own contribution

For the following publication, my primary contributions involved conducting the laboratory research and analysing the data and images. I independently carried out all experimental investigations and processed the resulting data to ensure the validity and accuracy of the results. My role also included collaborating with our co-operation partner Wencke Wegner in the preparation and visualization of the samples, ensuring high-quality imaging and accurate representation of the microplastic structures in the scanning electron microscopy. Together

with my supervisor Maja Henjakovic, I contributed to the statistical analysis to support the conclusions of the study and co-authored the original manuscript. Additionally, my responsibilities involved assisting in processing the editor's and reviewer's comments during the revision and resubmission process. Furthermore, I conducted a comprehensive literature review to provide context for our findings and support a robust scientific discussion.

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Testing of Different Digestion Solutions on Tissue Samples and the Effects of Used Potassium Hydroxide Solution on Polystyrene Microspheres

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Abstract: Microplastic particles are ubiquitous in our environment, having entered the air, the water, the soil, and ultimately our food chain. Owing to their small size, these particles can potentially enter the bloodstream and accumulate in the organs. To detect microplastics using existing methods, they must first be isolated. The aim of this study was to develop a non-destructive method for efficiently and affordably isolating plastic particles. We investigated the digestion of kidney, lung, liver, and brain samples from pigs. Kidney samples were analyzed using light microscopy after incubation with proteinase K, pepsin/pancreatin, and 10% potassium hydroxide (KOH) solution. Various KOH:tissue ratios were employed for the digestion of lung, liver, and brain samples. Additionally, we examined the effect of 10% KOH solution on added polystyrene microplastics using scanning electron microscopy. Our findings revealed that a 10% KOH solution is the most suitable for dissolving diverse organ samples, while enzymatic methods require further refinement. Moreover, we demonstrated that commonly used 1 µm polystyrene particles remain unaffected by 10% KOH solution even after 76 h of incubation. Digestion by KOH offers a simple and cost-effective approach for processing organ samples and holds potential for isolating plastic particles from meat products.

Keywords: microplastics; 1 µm polystyrene particles; organ samples; enzymatic and alkaline digestion; method development

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1. Introduction

Microplastic pollution is increasingly on the rise and an expanding shift from macro- to microparticles can be expected [1]. Two different sources for the formation of microplastics have been described. Primary microplastics are small plastic particles that are intentionally manufactured to be used in various products such as cosmetics (e.g., microbeads), cleaning products, and industrial abrasives (e.g., pellets and flakes) [2]. Secondary sources include the breakdown and fragmentation of larger plastic debris and the release of microplastics as a result of chemical, physical, and biological degradation processes [3]. Microplastics have been detected in various settings, including the air, oceans, soils, and, accordingly, food items [4–6]. Regardless of the source, the ingestion of microplastics can result in adverse effects on human health, triggering a series of biological responses [7].

Regarding the size definition for microplastics, an upper size limit of microplastic particles has been set at 5 mm [8], with 1 µm representing the lower limit [9], and particles of up to 0.1 µm are referred to as submicroplastics [10].

A recent review has emphasized that the prevalence of microplastics in the environment, in drinking water, and especially in human food presents a growing concern [11]. Therefore, developing a simple and efficient method for detecting microplastics in human

fluids, human tissue, and various food items, such as meat products, is of the utmost importance. There are already numerous methods for analyzing larger microplastic particles in environmental samples. Microscopy is a commonly used method that allows the visual identification and quantification of microplastics [12].

In order to be able to identify plastic particles using existing spectrometric techniques such as infrared and micro-Raman spectroscopy, as well as mass-based techniques like Pyrolysis-GC-MS, it is necessary to isolate the particles from the biological matrix [10]. Environmental scientists have developed a variety of techniques for separating plastic from organic material, such as sewage sludge, sediment, and water. The most commonly used methods involve acidic and alkaline treatments, although some researchers have also explored enzymatic approaches [13–15]. Different experimental setups with similar chemical or enzymatic digestion mixtures have been used for human or animal blood or tissue. Leslie et al. used enzymatic digestion with proteinase K to detect microplastics in the blood for the first time [16].

Nano- and microplastic particles can accumulate in the organs or be cleared into interstitial space due to the cross-linking of vessels, resulting in a reduction in vessel lumina. This has been confirmed with the detection of plastic particles in various human organs. Recent studies have demonstrated the presence of microplastics in the lungs [17] as well as in the placenta [18]. Similarly to what has already been observed in humans, the accumulation of microplastics in the organs of farm animals, such as pigs, cattle, and poultry, is also possible [19]. In experimental settings, the accumulation of plastic has also been detected in various tissues of mice, such as the kidney, lung, liver, and brain. Plastic accumulation and toxicological effects were detected in these animal experiments after application to tissues [20,21]. However, the application amount was partially higher than the physiological concentration that has already been detected in human tissues.

The objective of this study was to adapt and optimize a rapid and efficient protocol for digesting various organ samples without destroying microplastic particles. For this purpose, enzymatic (proteinase K, pepsin, and pancreatin) protocols were compared to an alkaline (10% potassium hydroxide, KOH) digestion treatment on different porcine tissues. These tissues were chosen because they are highly similar to human tissues, and the internal organs of the pig, like the liver, are frequently consumed or used as ingredients in meat specialties.

The uniqueness of proteinase K lies in its ability to degrade a wide range of proteins, including those with stable or hard-to-reach structures. For this reason, proteinase K is employed in numerous molecular biological and histological procedures and has also been used for isolating microplastics from human blood [16]. The objective here was to investigate whether the proteinase K protocol is suitable for the complete digestion of various porcine organs.

In biomedical research, various protocols have been developed for in vitro digestion utilizing the digestive enzymes pepsin and pancreatin. Examples include the digestion of microalgae, bovine, and porcine muscle tissue [22–24]. The aim of this study was to employ a straightforward in vitro digestion protocol with pepsin and pancreatin to achieve the complete digestion of porcine organs.

For the isolation of plastic particles from environmental, animal, and human samples, acids and alkalis have frequently been employed, providing a simple and cost-effective method. A 10% KOH (1.8 M) solution, for instance, has been utilized for the dissolution of human placentas [18]. Some studies have indicated that a 10% KOH solution will reduce the recovery of certain plastics, such as polycarbonate, polyvinyl chloride, and polyethylene terephthalate, while others have ruled out degrading effects at milder temperatures [25–28]. Following extensive literature research that took into account observed effects on various types of plastics, as well as the conduction of numerous preliminary experiments, the alkaline dissolution of kidney, liver, lung, and brain samples in a 10% KOH solution at 37 °C was planned for investigation.

Additionally, the effects of KOH on 5 µm and especially 1 µm polystyrene (PS) microspheres were examined under specific experimental conditions. These model particles were roughly the size of microplastic particles that can circulate in the bloodstream. Polystyrene particles have been recognized as one of the most encountered plastics in human blood [16], are commonly utilized in research studies [20,21], and, due to their extensive surface area, offer a substantial site of interaction for reagents, including the KOH solution in this case. Viewed critically, this does not permit the exclusion of the effects of KOH on various types of plastic particles; it exclusively addresses alterations in PS microspheres. Moreover, the impact of KOH on PS particles actually present in organs could yield different outcomes depending on their properties.

2. Materials and Methods

2.1. Sample Collection

Human blood samples were acquired from four anonymized healthy donors, comprising one female and three males aged between 22 and 26 years. The samples were collected using 10 mL EDTA Vacutainers (367525, BD Biosciences, Vienna, Austria). Venipuncture was performed with a sterile Vacutainer-Safety-Lok 21G (367282, BD Biosiences). Immediately after sampling, the blood samples were stored in a −20 °C freezer until analysis.

Porcine tissue, including kidney, liver, lung, and brain tissue, was obtained from a local butcher. Upon arrival, the porcine tissue was carefully washed and then cut into smaller portions under sterile conditions to prevent contamination. Each tissue portion was then stored at −20 °C until further use.

2.2. Polystyrene (PS) Polymer Microspheres

The used polystyrene (PS) polymer microspheres with sizes of 1 µm and 5 µm had no surface modifications (BS-Partikel GmbH, Mainz, Germany; HS0100-20 and HS0500-20, respectively). These microspheres were produced by the manufacturer using standardized methods and underwent characterization. For the 1 µm microspheres, the manufacturer specified a mean diameter of 0.988 µm with a relative standard deviation (CV) of 2.2%, while the 5 µm microspheres had a mean diameter of 4.96 µm with a CV of 1.6%. The microspheres were composed of poly(styrene-co-divinylbenzene), possessed a density of 1.05 g/cm^3, and exhibited a refractive index of 1.59 at 25 °C.

2.3. Microscopy

A light microscope (Leica DM2500 LED, Leica Microsystems AG, Balgach, Switzerland) was used for the visualization of alkaline and enzyme-dependent digestion and the counting of polystyrene (PS) plastic particles. Unless otherwise stated, pictures were taken with fortyfold magnification.

Scanning electron microscopy (SEM) was performed at the Central Research Laboratories of the Natural History Museum of Vienna (NHMW). The samples were first coated with platinum (Leica EM CSD 500, Leica Microsystems AG, Balgach, Switzerland) and subsequently visualized with a JEOL JSM-6610 (JEOL AG, Freising, Germany) at an accelerating voltage of 20 kV and a spot size of 40.

2.4. Digestion

2.4.1. Enzymatic Digestion Method Using Proteinase K

For enzymatic digestion using proteinase K, the digestion protocol for blood described by Leslie et al. was adopted [16]. The frozen tissue (kidney, lung, liver, brain) was mechanically processed using a mortar until a homogeneous pulpy consistency was achieved. The amount of tissue was adjusted to the hematocrit value. Accordingly, 0.45 g of homogenized tissue, which approximately corresponded to the cellular component of 1 g of blood, was used. Additionally, a control setup was performed with 1 g of blood.

Then, 15 mL of Tris-HCl buffer (400 mM Tris-HCL; J22638.K2; Thermo Fisher Scientific, Vienna, Austria; pH 8; 0.5% SDS ultrapure; 2326.1; Carl Roth GmbH + Co. KG, Karlsruhe,

Germany) was added per 1 g of blood or 0.45 g of homogenized tissue and incubated in a water bath at 60 °C for 1 h to denature the proteins. For further digestion, 100 µL of proteinase K (1 mg/mL; ≥3.0 unit/mg solid; P8044; Sigma-Aldrich Handels Gmbh, Vienna, Austria) was inserted together with 1 mL of 5 mM calcium chlorite ($CaCl_2$; HN04.2; Carl Roth) and incubated for another 2 h at 50 °C. The flask containing these elements was then shaken for 20 min at room temperature and finally heated in a water bath to 60 °C for another 20 min.

The effects of proteinase K on blood and tissue samples were investigated using light microscopy (Leica DM2500 LED, Leica Microsystems AG, Balgach, Switzerland) in a minimum of ten pictures, and the amount of cell debris was determined using Fiji (ImageJ, version 2.1.0/1.53c; National Institutes of Health). In order to compare the results of the blood digestion with proteinase K with the conditions of an initial time point, the blood was diluted with PBS according to the work volume in the proteinase K digestion protocol.

2.4.2. Enzymatic Digestion Method Using Pepsin and Pancreatin

The porcine pepsin (KK38.1; Carl Roth GmbH + Co. KG, Karlsruhe, Germany) had a specific enzyme activity of ≥2000 FIP-U/g as provided by the manufacturer. For the stock solution, the pepsin was dissolved in 10 mM HCl to obtain a concentration of 2 mg/mL.

The porcine pancreatin (A0585; AppliChem GmbH, Darmstadt, Germany) had a specific enzyme activity of 36,000 FIP-U/g as provided by the manufacturer. For the stock solution, the pancreatin was dissolved in Dulbecco's phosphate-buffered saline (DPBS; 14190-144; Thermo Fisher Scientific) to obtain a concentration of 2 mg/mL.

The porcine kidney, lung, liver, and brain tissue was mechanically processed using a mortar until a homogeneous pulpy consistency was achieved. An amount of 0.45 g of homogenized tissue was used for each experiment.

The first step of the enzymatic digestion protocol was the denaturation and pre-digestion of the homogenized tissue by hydrochloric acid (HCl) and pepsin. For the optimal activity of the pepsin, homogenized tissue with an added HCl solution (160 mM in end volume) was mixed in 100 mL Erlenmayer flasks and pre-incubated in a water bath for 5 min at 37 °C before 2 mg of pepsin was added. The mixture was incubated for 4 h at 37 °C in a water bath with shaking.

Subsequently, the mixture was buffered to a pH between 7 and 7.4 by adding 1 M sodium hydrogen carbonate ($NaHCO_3$; 8551.1; Carl Roth GmbH). After neutralization, 20 mg of pancreatin was added as a single dose and incubated for 24.5 h.

Enzymatic digestion was investigated using light microscopy (Leica DM2500 LED) after a 4 h incubation time for pepsin and a 24.5 h incubation time for pancreatin, with a minimum of twenty pictures across two cover slips per sample. The amount of cell debris was determined using Fiji (ImageJ, version 2.1.0/1.53c; National Institutes of Health).

2.4.3. Alkaline Digestion Method

For alkaline digestion, porcine organs (kidney, lung, liver, brain) were treated with 10% (1.8 M) potassium hydroxide solution (KOH; P747.1; Carl Roth). The ratios of sample to alkaline reagent used were 1:4 (w/v) for the first experimental approach and 1:8 (w/v) for a further tissue digestion trial. First, KOH solutions were transferred to autoclaved 100 mL Erlenmeyer flasks; then, small-cut pieces of different tissues were added. The flasks were incubated in a water bath at 37 °C for 76 h. The digestion progress was checked using light microscopy at the time points of 0 h (immediately after preparing the mixture), 6 h, 24 h, and 76 h for the 1:4 (w/v) ratio. An evaluation of the digestive process was conducted solely after durations of 6 h and 76 h for the 1:8 (w/v) ratio.

The effects of alkaline reagents on various porcine tissues were investigated using light microscopy (Leica DM2500 LED) in a minimum of twenty pictures across two cover slips per sample, and the amount of cell debris was determined using Fiji (ImageJ, version 2.1.0/1.53c; National Institutes of Health).

2.5. Effects of Digestion Protocols on Plastic Particles

In addition, the effect of 10% KOH digestion solution on 1 µm and 5 µm polystyrene (PS) particles was examined.

For this purpose, 1 µm and 5 µm PS particles were added to alkaline digestion solution. To prevent the particles from being obscured by digestive residues or aggregating to them, the tissue samples were replaced with H_2O and 1 µm particles were investigated at a 1:4 ratio, while 5 µm particles were investigated at a 1:8 ratio with KOH. Only particle-free water was used for the alkaline control. The solutions were then incubated for 76 h at 37 °C. Light microscopy was used to directly examine the particles in the incubation solution at different time points between 0 h (immediately after preparing the mixture) and 76 h.

In addition, effects on the size as well as changes in the surface structure of the 1 µm isolated particles from the alkaline digestion solution and the alkaline control group were investigated with SEM (JEOL JSM-6610). For this purpose, the samples were subsequently filtered using 0.45 µm track-etched polycarbonate membrane filters (A046.1; Carl Roth). The filters were washed three times with 15 mL of particle-free H_2O, dried, and then affixed to the corresponding SEM carbon tape.

2.6. Statistical Analysis

All data are represented as means ± SD. Statistical analyses were performed using a two-tailed unpaired *t*-test when testing between two time points, and one-way analysis of variance with Dunnett's multiple comparison test was performed for within-group comparisons (GraphPad Prism9, version 9.3.1). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Kidney, Lung, Liver, and Brain Tissue Digestion Using Proteinase K

The enzymatic digestion method with proteinase K was applied to homogenized kidney, lung, liver, and brain tissue according to the protocol of Leslie et al. [16]. Almost no digestive progress could be observed when 1 g of kidney tissue was used. In reducing the amount of tissue to approximately the hematocrit value of the blood, slight digestive progress could be seen. Figure 1A shows that fewer kidney tissue residues were visible after complete proteinase K digestion compared to protein denaturation before proteinase K. However, even with these adjustments, sufficient digestion was not yet to be observed. Similarly, the lung, liver, and brain samples exhibited only minimal digestion upon treatment with proteinase K (Figure S1). Blood digestion with proteinase K gives very good and highly significant results, as can be seen in the experiment of Leslie et al. [16] and in our experiment (Figure 1B). Compared with an adequately diluted blood sample in PBS, only some, almost invisible, cellular debris remained after the digestion with proteinase K (Figure 1B).

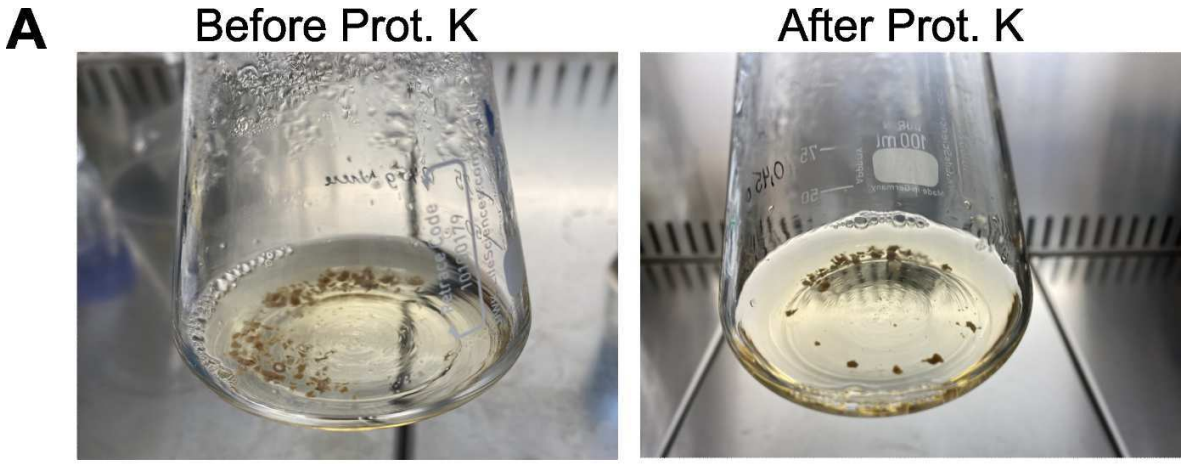

Figure 1. *Cont.*

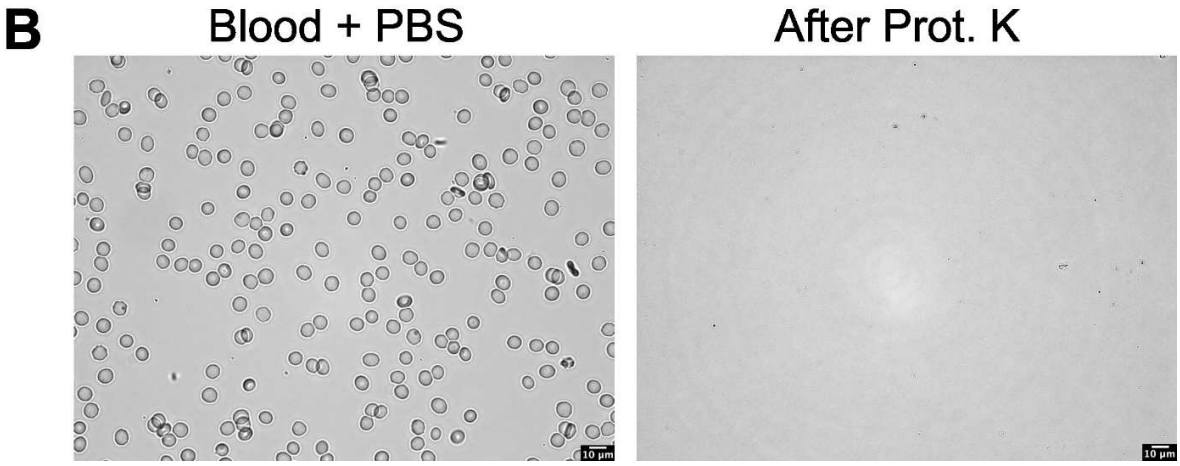

Figure 1. Enzymatic digestion of porcine kidney samples (**A**) and blood (**B**) using proteinase K (Prot. K). (**A**) These images depict homogenized kidney tissue in the digestion solution before and after incubation in proteinase K, as microscopic examination was not feasible. (**B**) These representative microscopic images show a blood sample diluted in PBS and after digestion with Proteinase K.

3.2. Kidney, Lung, Liver, and Brain Tissue Digestion Using Pepsin and Pancreatin

As a further manner of tissue digestion, an enzymatic method utilizing pepsin and pancreatin was employed. After incubation with pepsin, the full amount of pancreatin was added, and microscopic images were taken at specific time intervals. There was one microscopic time point 4 h after the addition of the pepsin, and a second microscopic time point was performed 24.5 h after the complete addition of the pancreatin.

After 4 h of incubation of homogenized kidney tissue with pepsin, large areas of cell debris remained visible (Figure 2A). However, after pancreatin incubation, the kidney tissue exhibited the presence of conspicuous large cell debris, which remained observable under microscopic examination (Figure 2A).

Only minimal differences were observed between the effects of pepsin and pancreatin on kidney digestion (Figure 2B). This observation contrasts with Figure 2A, in which the undigested area appears to have decreased markedly after 24.5 h of incubation with pancreatin. The reason for this apparent inconsistency is that in Figure 2B, the value of cell debris for incubation in pancreatin has been corrected by a factor of 5.7 to account for the greater volume during pancreatin digestion compared with that after 4 h of pepsin incubation. Similarly, it was not possible to digest lung, liver, or brain tissues using pepsin or pancreatin (Figure S2).

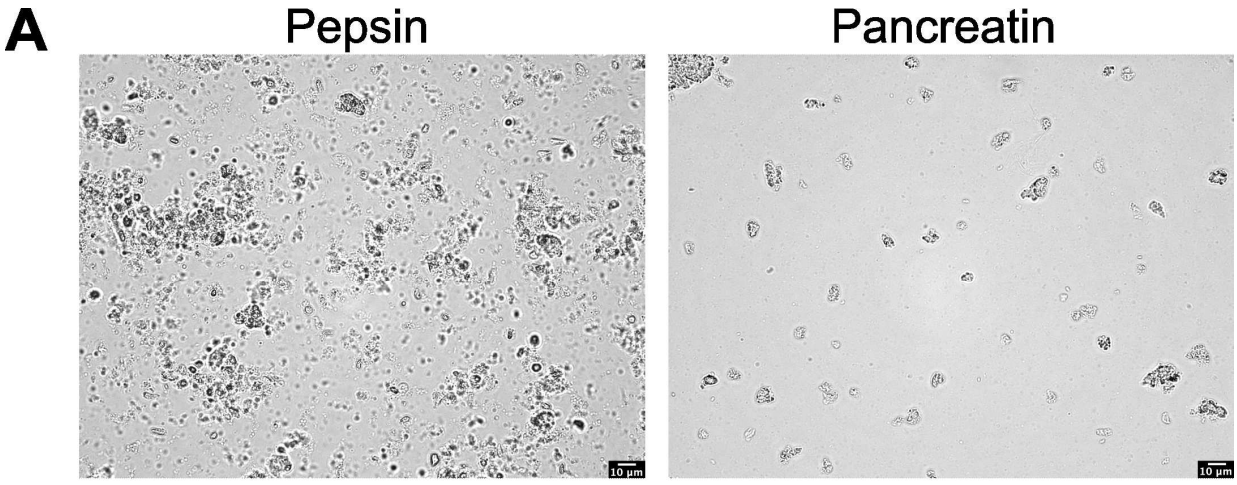

Figure 2. *Cont.*

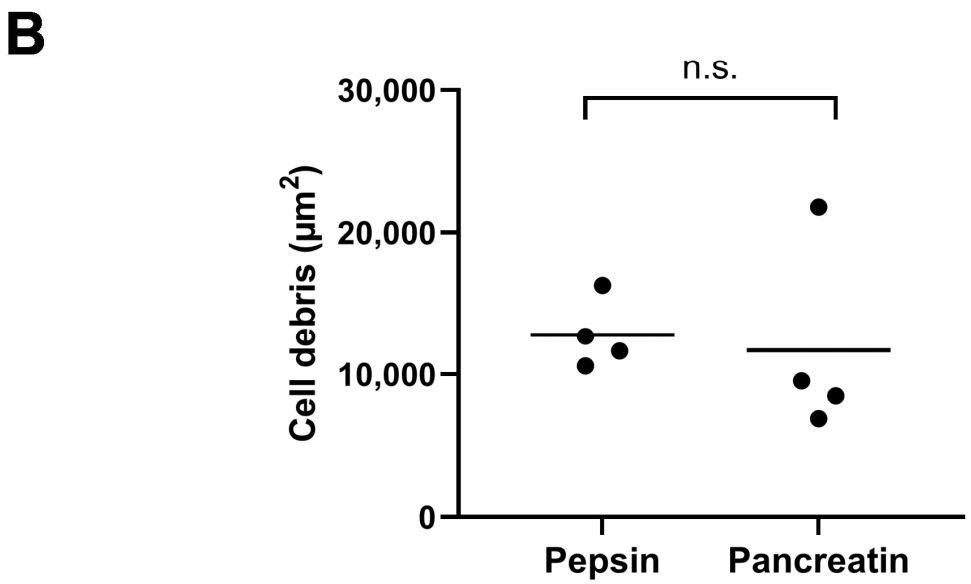

Figure 2. Enzymatic digestion of kidney tissue using pepsin and pancreatin. The kidney homogenates were incubated for 4 h in a 2 mg/mL pepsin solution, followed by an additional 24.5 h in a 2 mg/mL pancreatin solution at 37 °C. (**A**) These representative microscopic images show the progress of digestion after incubation in pepsin and after subsequent incubation in pancreatin. (**B**) Cell debris was detected with light microscopy, and subsequent image analysis used a Fiji macro. Data points represent experimental repetition, and the crossbar represents the mean. $n = 4$. Statistical significance was determined using a two-tailed unpaired *t*-test. n.s.: not significant.

3.3. Kidney, Lung, Liver, and Brain Tissue Digestion Using KOH

In addition, an alkaline method using potassium hydroxide (KOH) was tested to verify the reliable and sufficient digestion of kidney tissue. Therefore, small kidney tissue pieces were first treated with a 10% KOH solution in a 1:4 (w/v) ratio, and the amount of cell debris was analyzed using light microscopy. Figure 3 shows that a highly significant progress in digestion could already be seen after 6 h. After 76 h, the area of undigested cell debris had already decreased to 11.3 ± 4.6% compared to the kidney tissue section, which can be assumed to have been at 100 ± 9.1%.

Due to the fact that the digestion provided good results for the kidney tissue, further tissues, such as the lung, liver, and brain, were also tested at a 1:4 (w/v) tissue-to-KOH ratio.

Both the lung tissue and the liver tissue showed similarly good results compared to the kidney tissue, with undigested cell remains areas of 14.2 ± 1.4% and 13.2 ± 2.1%, respectively (Figure 3). However, in the case of the lung tissue, after 6 h, there were still small tissue fragments visible in the solution, which is why the microscopic analysis was not considered meaningful at that point. The brain tissue provided the worst digestion results. Even after 76 h of incubation in 10% KOH solution, the sample showed 62.2 ± 16.2% of undigested residues, which indicated no significant differences compared to the brain tissue section (100 ± 27.9%).

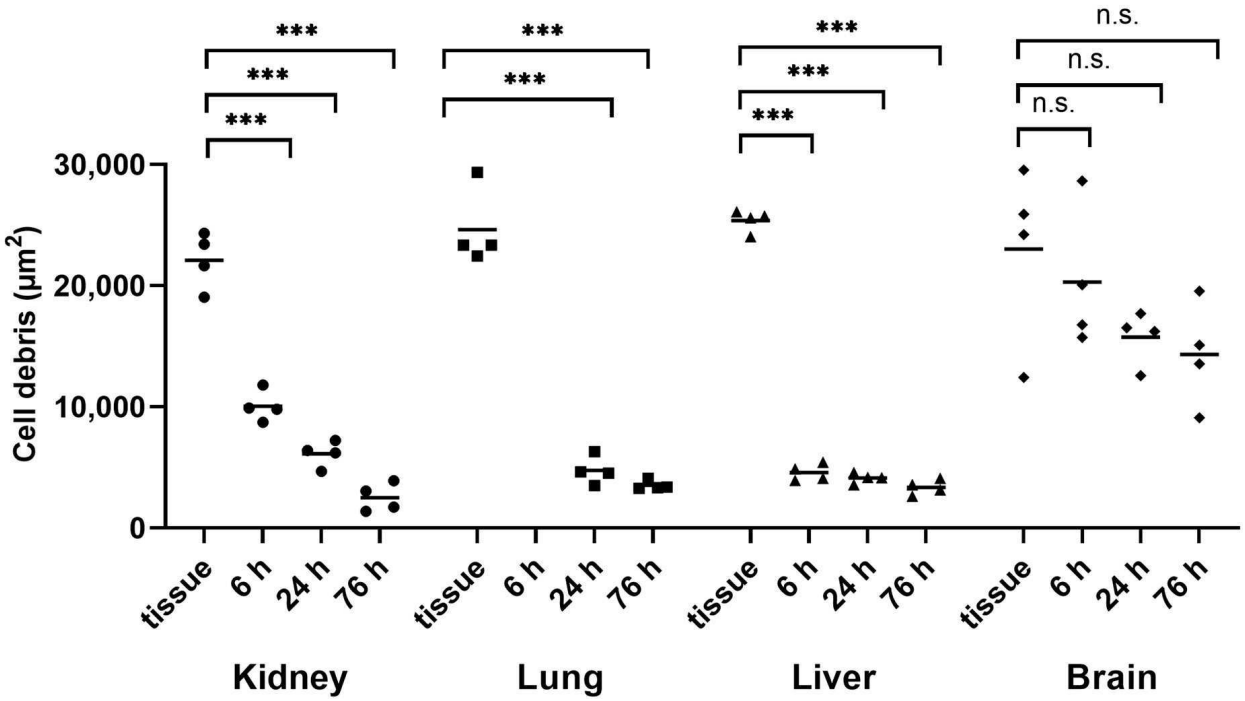

Figure 3. Time-dependent alkaline digestion of tissue samples using 10% KOH at a ratio of 1:4 (*w*/*v*) and an incubation temperature of 37 °C. This graphic illustration shows kidney, lung, liver, and brain tissue digestion. Cell debris was detected with light microscopy, and subsequent image analysis was performed with a Fiji macro. Data points represent experimental repetition, and the crossbar represents the mean. $n = 4$. Statistical significance was determined using a one-way analysis of variance with Dunnett's multiple comparison test for within-group comparisons (20 µm thickened cryostat versus 6 h, 24 h and 76 h effect of 10% KOH solution on tissue samples). n.s.: not significant; ***: $p < 0.001$.

3.4. Effect of 10% KOH Solution on Polystyrene (PS) Microspheres

The effect of the KOH solution on 1 µm polystyrene particles (PS) was tested. For this experiment, the 1 µm PS particles were incubated in 10% KOH solution and in H_2O as control attempt for 0 h, 24 h, and 76 h at 37 °C. However, at 37 °C, the used 1 µm particles in the KOH did not show any significant differences (Figure 4).

In addition, PS particles were incubated in 10% KOH, and their alteration was investigated with scanning electron microscopy (SEM). In order to obtain an overview of the appearance and behavior of the particles that adhered to the carbon tape, 1 µm PS plastic particles incubated in H_2O for 76 h at 37 °C were examined at ten-thousandfold magnification. It seemed that the particles did not all have the same size, due to the fact that they were located in different plains. Figure 5 shows SEM views of the 1 µm PS microplastic particles, in which ten different particles (yellow numbers 1–10) were selected and measured. The particle size range was 0.94 ± 0.07 µm and the particles were spherical with smooth surfaces (Figure 5). It was found that both larger particles and smaller particles were captured in the image (Figure 5). The main focus was probably on the particle with localization number 3 (Figure 5).

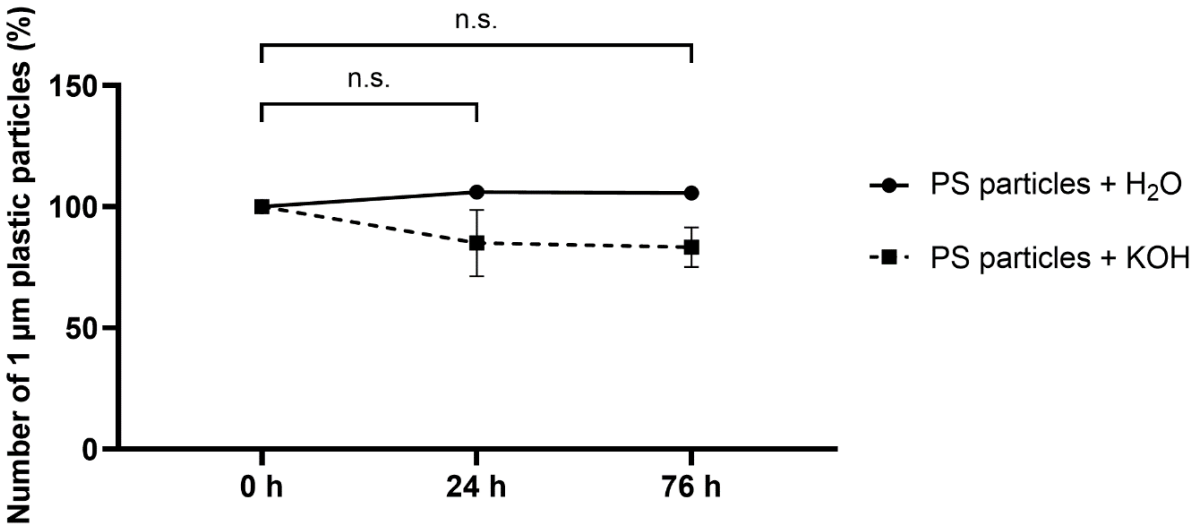

Figure 4. Effect of KOH solution on 1 µm polystyrene (PS) plastic particles. PS particles were incubated with 10% KOH solution in the absence of human blood and in an H_2O control at 37 °C for up to 76 h. They were counted using the Fiji cell counter tool. Data represent means ± SD. n = 3 per treatment. n.s.: not significant; within the KOH group in comparison with 0 h.

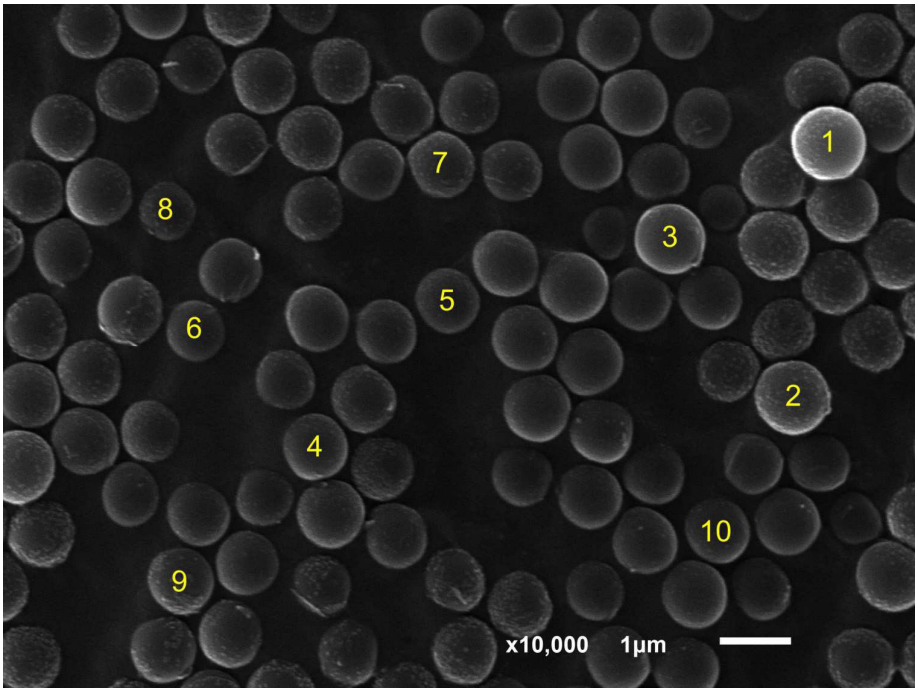

Figure 5. Overview image from scanning electron microscopy (SEM) showing 1 µm PS particles incubated in H_2O at 37 °C for 76 h. Particles were captured at ten-thousandfold magnification.

To investigate potential surface or size alterations in 1 µm PS particles after 76 h incubation in 10% KOH at 37 °C, SEM was conducted. The images of the KOH-treated particles were compared with images of those that underwent incubation in H_2O. Several images of both experimental setups, each with a sample size of 4 (n = 4), were captured. Figure 6 displays a representative subset of these images, taken at magnifications of twenty-thousandfold and thirty-five-thousandfold. There were no differences between the H_2O and the KOH solution (Figure 6). The size distributions of the particles were similar

in both solutions, and the surfaces of the particles did not indicate any alterations in either case (Figure 6).

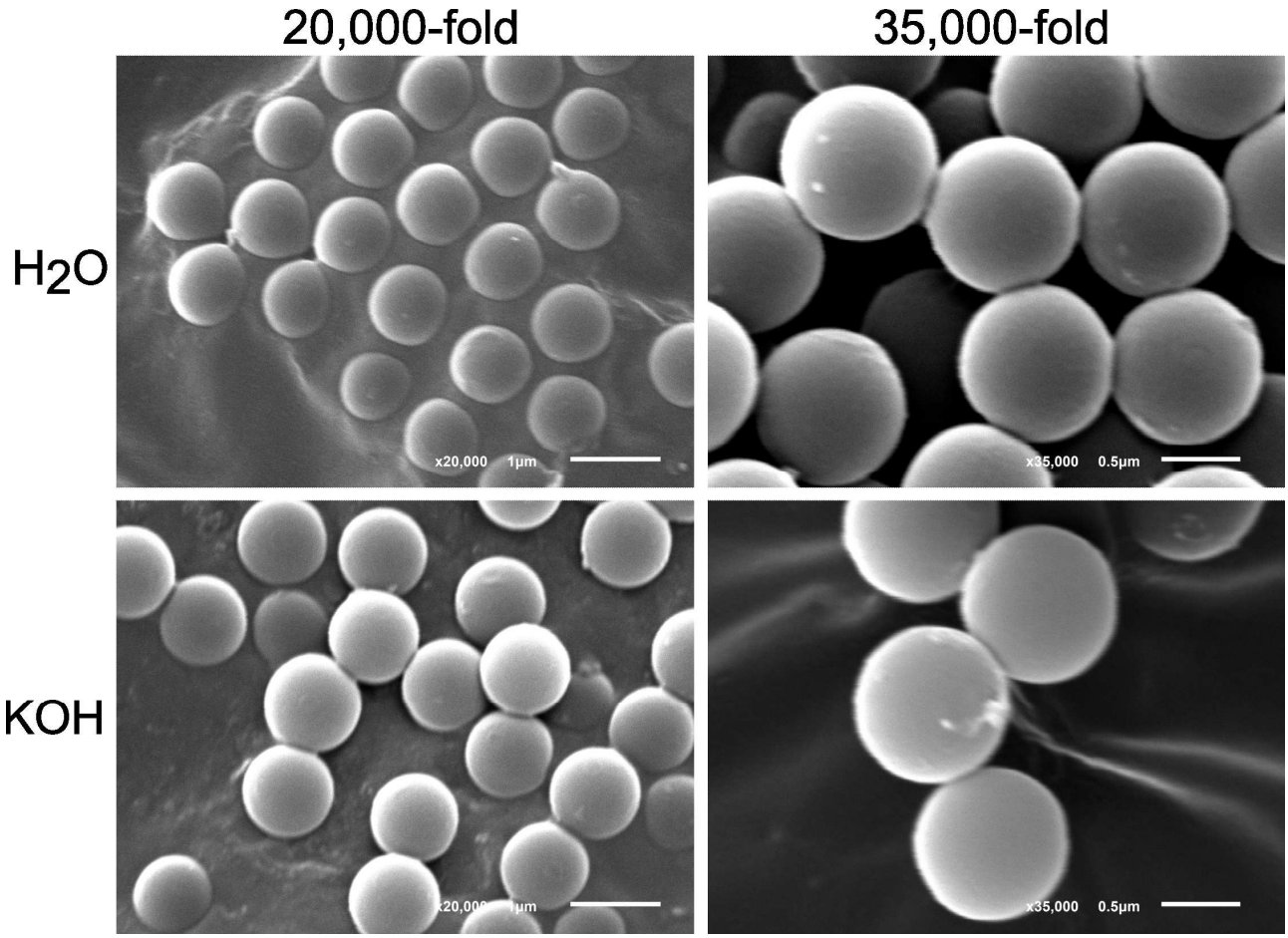

Figure 6. Scanning electron microscopy (SEM) of 1 µm particles after 76 h of incubation at 37 °C in H_2O (**top**) and in 10% KOH solution (**bottom**) and representative images of 1 µm PS particles with twenty-thousandfold (**left**) and thirty-five-thousandfold (**right**) magnification.

In order to check whether an increase in KOH would yield better digestion progress, the tissue-to-KOH ratio was increased to 1:8 (w/v) according to a digestion protocol set by Ragusa et al. [18].

As depicted in Figure 7, the 1:8 (w/v) tissue-to-KOH ratio appeared to improve the digestive progress in all four tissues when compared with the 1:4 (w/v) ratio. However, when the dilution factor was included in the calculation, no improvement in the progress of digestion could be seen in any of the tissues (Figure 8). Figure 8 further shows that the significances remained the same as in Figure 3 with the tissue-to-KOH ratio of 1:4 (w/v). Even for the brain tissue, no improvement in digestion could be seen (Figures 3 and 8).

To investigate potential alterations in 5 µm PS particles under varying concentrations of KOH, the particles were subjected to incubation in water instead of tissue, along with a 10% KOH solution, at a ratio of 1:8 (w/v). No changes in the number of particles could be detected between the observation times of 0 h (examination directly after the addition of particles) and 76 h (Figure 9). Furthermore, the figure shows that there were no changes in the surfaces or shapes of the particles.

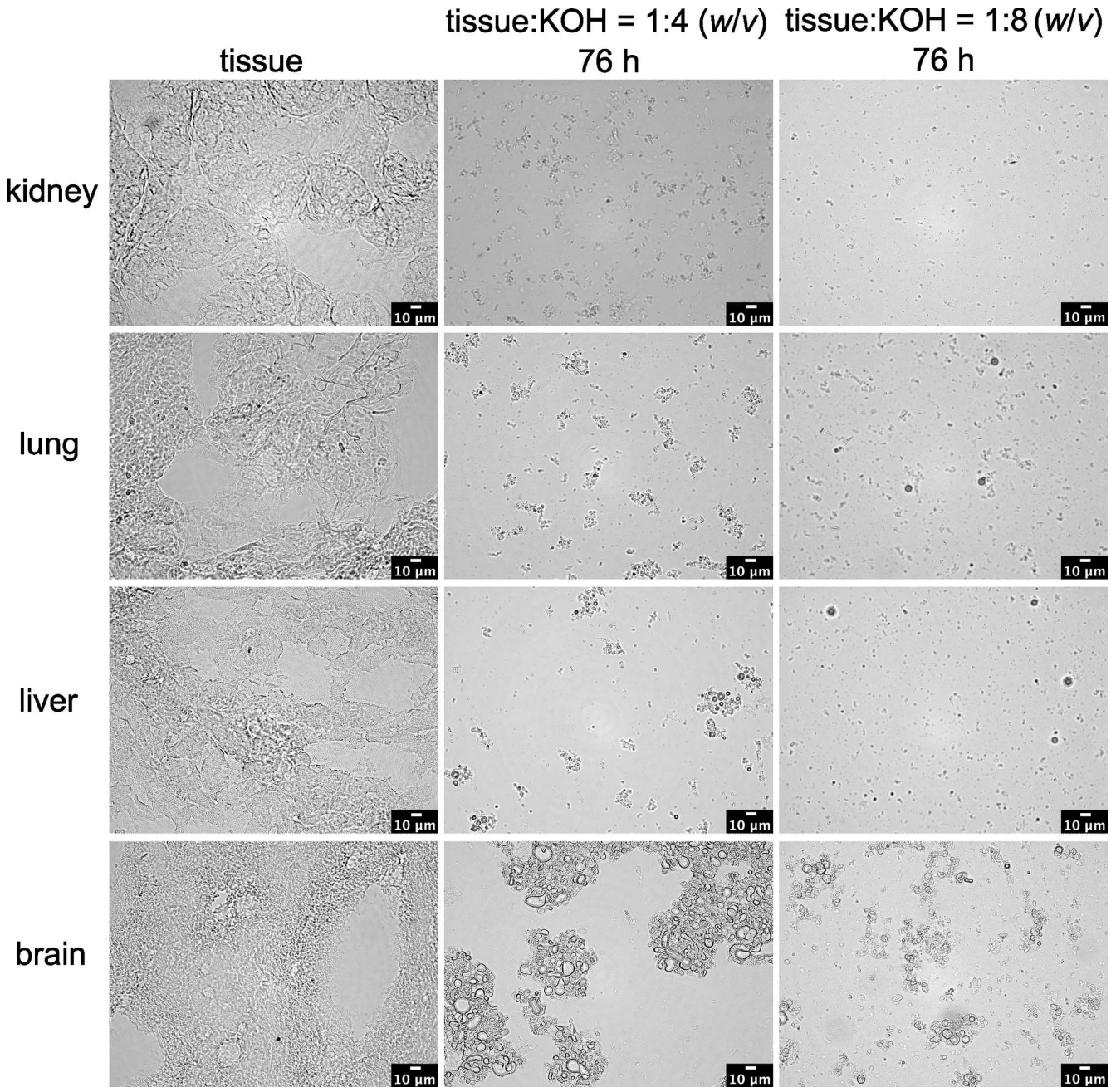

Figure 7. Alkaline digestion of four different tissue samples using 10% KOH solution at an incubation temperature of 37 °C. These representative microscopic images show the comparison between tissue sections (20 µm) of the kidney, lung, liver, and brain and digestion using tissue-to-KOH ratios of 1:4 (*w*/*v*) and 1:8 (*w*/*v*), respectively, after 76 h.

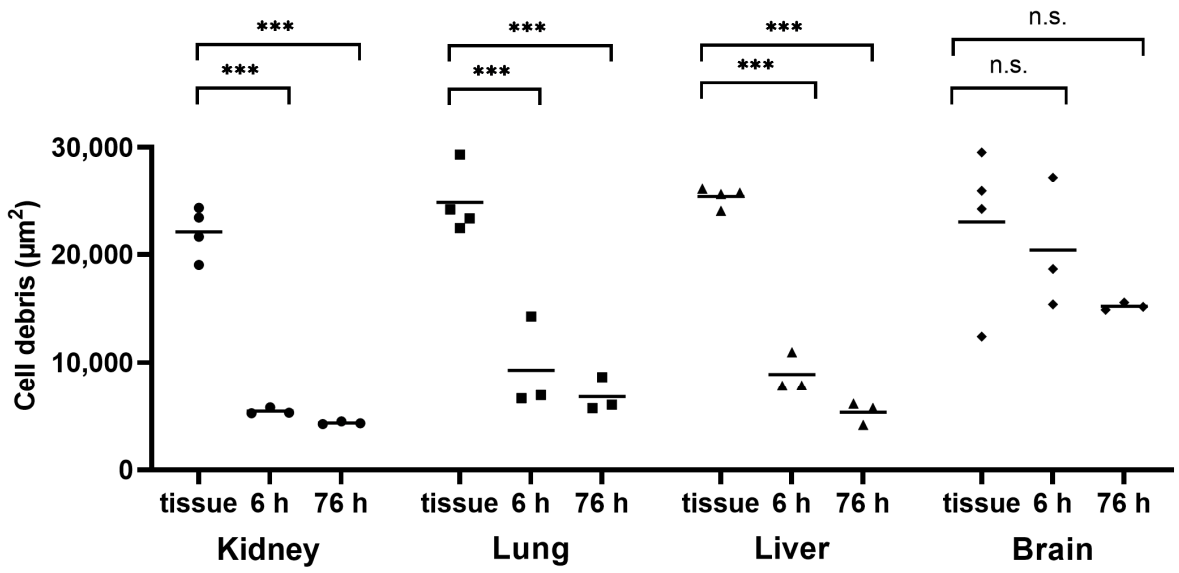

Figure 8. Time-dependent alkaline digestion of tissue samples using 10% KOH solution at a ratio of 1:8 (*w*/*v*) and an incubation temperature of 37 °C. This graphic illustration shows kidney, lung, liver, and brain tissue digestion. The results for the 1:8 dilution ratio were adjusted to the 1:4 dilution ratio used in the previous experiment. Cell debris was detected with light microscopy and subsequent image analysis was performed with a Fiji macro. Data points represent experimental repetition and the crossbar represents the mean. $n = 4$. Statistical significance was determined using a one-way analysis of variance with Dunnett's multiple comparison test for within-group comparisons (20 µm thickened cryostat versus 6 h and 76 h incubation). n.s.: not significant; ***: $p < 0.001$.

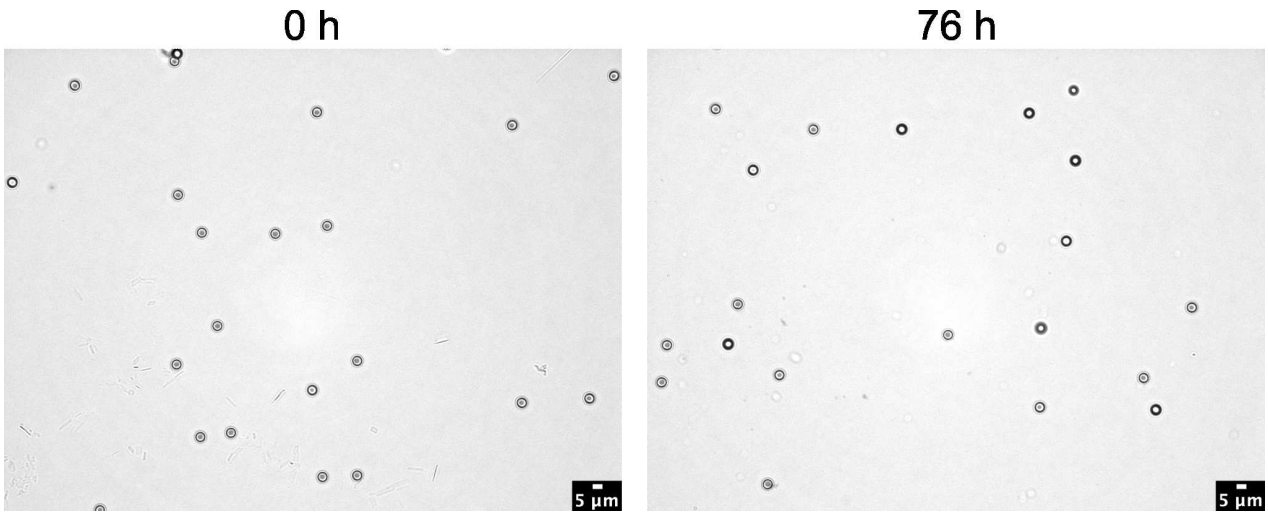

Figure 9. Light microscopic images of the 5 µm particles incubated in water instead of kidney tissue along with 10% KOH at a ratio of 1:8 (*w*/*v*). These representative images were taken after an incubation period of 0 h to 76 h.

4. Discussion

The aim of this study was to identify a suitable method for effectively digesting various animal tissues to enable subsequent microplastic determination and analysis. Various digestion reagents and protocols were employed to establish a simple and effective method for digesting animal tissue.

The successful application of proteinase K digestion for the isolation and quantification of microplastics in marine organisms has been demonstrated in a marine environmental study [13]. By using this digestion method, Cole et al. were able to overcome the challenge

of isolating microplastics from complex, biota-rich seawater samples and marine organisms by achieving a digestion efficiency of >97%. In the course of this study, a digestion protocol that has already been established for human blood samples was used [16]. However, problems were encountered when this protocol was applied to different types of porcine tissue, even though satisfactory digestion results were obtained when the same digestion protocol was applied to human blood samples as a control experiment. This limitation impacted the efficacy of assessing the behavior of plastic particles within the digestive solution. Possible reasons may include that the incubation time and proteinase K concentration used in this experiment were not sufficient for optimal digestion. Furthermore, employing a comprehensive cellular-level homogenization technique or extending the denaturation period before adding proteinase K could potentially yield improved results. However, a publication by Liang et al. offers a potential solution of using proteinase K in a different buffer solution than Tris-HCl, which might give better results [29]. Another study has described optimum digestion for freshwater snails using a mixture of Tris-HCl, proteinase K, and potassium hydroxide (KOH) [30]. The inclusion of KOH can enhance digestion efficiency by facilitating the dissolution of organic matter, leading to improved recovery of microplastics. Additionally, it has been described that these mild digestion conditions do not alter contained microplastics, and therefore, this method is also well-suited for extracting microplastics from tissue [30].

While various digestion protocols have been successfully applied for proteinase K, there is currently no established protocol for organ tissue digestion using the enzymes pepsin or pancreatin based on the current available knowledge and understanding. However, both pepsin and pancreatin have the potential to be utilized for tissue digestion due to their enzymatic properties and functional relevance in the human digestive system. Pepsin, a proteolytic enzyme produced in the stomach, is essential for the breakdown of proteins during the digestive process [31]. Pancreatin, on the other hand, holds promise for tissue digestion by facilitating the breakdown of various components within the tissue, including proteins, fats, and carbohydrates [32]. The digestion protocol with pepsin and pancreatin used in this study resulted in inefficient tissue digestion. Therefore, it was not possible to isolate plastic particles from the organs for quantitative or qualitative analysis. Further research and experimentation are needed to develop effective and reproducible protocols for tissue digestion with pepsin and pancreatin. Thus, increasing the concentrations of the enzymes used or the subsequent addition of enzymes such as trypsin could improve digestion efficiency. In addition, alternative pepsin and pancreatin solutions with higher enzymatic activity, as indicated by the manufacturer, could be investigated in future studies. However, the implementation of these possible optimizations would involve more work and higher costs. Enzymatic digestion is very promising for the isolation of plastic particles from different samples. Nevertheless, given the fascinating physiological processes in the gastrointestinal tract, it is likely that the experimental conditions, such as the concentration of enzymes, will need to be adapted for each sample type.

As a further digestive solution, 10% potassium hydroxide (KOH) was used because it is a commonly used and very simple and effective digestion method for a wide variety of organic materials, like marine samples, animal tissue, and human tissue [18,33–35].

After achievement of the successful dissolution of kidney tissue under the selected test conditions, the digestion of other pig organs was examined. The findings of this study revealed that lung and liver tissue were effectively dissolved using a 10% KOH solution after 76 h at 37 °C, while brain tissue was not. The inefficient dissolution of brain tissue is likely attributed to its high lipid content, which may necessitate the use or addition of organic solvents like methanol/chloroform and should be explored in future investigations [36,37].

KOH is frequently employed in the context of microplastic analysis, wherein thorough examination of the effects of this strong alkaline solution on microplastic particles is to ensure that these digestion conditions do not affect the structural and chemical characteristics of the plastic particles.

Considering the extensive use of polystyrene (PS) particles found in various studies on microplastics in animals [30,38–41] and their easy accessibility and availability on the market at a low cost, the effects of a 10% KOH solution on 1 µm PS particles at 37 °C for 76 h were examined. Moreover, the presence of polystyrene particles has been identified in human blood [16], suggesting that plastic particles of this size may also be relevant in human and animal organs. Light microscopy analysis of the particle solution revealed no significant change in the particle count after KOH incubation, while electron microscopy confirmed no alteration in particle size or surface area. These results align with previous findings by Gulizia et al., who observed no impact of KOH on PS particles even at extreme temperatures [42]. However, other studies have reported potential effects of KOH on PS particles under different experimental conditions, recommending, for instance, incubation at a maximum of 40 °C [43]. Additionally, it is important to consider the possibility of interactions with other plastics when utilizing a 10% KOH solution, as previously described [26].

To assess the impact of 10% KOH solution on digestion efficiency, we increased the tissue:KOH ratio from 1:4 to 1:8, as previously employed for placenta dissolution [18]. However, even after consideration of dilution, no significant enhancement in tissue digestion was observed in this study. Furthermore, the quantity of 5 µm PS particles, easily detectable through light microscopy, remained unaffected by the increased amount of KOH solution.

5. Conclusions

The findings of this study indicate the need for further optimization of enzymatic procedures with proteinase K and a pepsin–pancreatin combination for tissue sample digestion, which may vary depending on the tissue type. The goal of optimizing and simplifying enzymatic digestion remains important, as it would enable the gentle isolation of plastic particles with varying sizes and compositions.

Additionally, this study demonstrated that complex organ tissues such as the kidneys, lungs, and liver can be effectively dissolved after incubation in a 10% KOH solution. This opens up possibilities for future investigations of the accumulation of plastic particles in these organs. It is particularly noteworthy that 1 µm polystyrene particles remained unchanged under the selected experimental conditions.

Considering the simplicity and cost-effectiveness of using 10% KOH solution for digestion, as well as its successful resolution of three out of four tested sample types, we recommend its application with the necessary further investigation of KOH's effects on different plastic particles.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/toxics11090790/s1, Figure S1. Enzymatic digestion of porcine lung, liver and brain samples using proteinase K (Prot. K). The images depict homogenized lung, liver and brain tissue in the digestion solution before and after incubation in proteinase K, as microscopic examination was not feasible; Figure S2. Enzymatic digestion of lung, liver and brain tissue using pepsin and pancreatin. The kidney homogenates were incubated for 4 h in a 2 mg/mL pepsin solution, followed by an additional 24.5 h in a 2 mg/mL pancreatin solution at 37 °C. Representative microscopic images show the progress of digestion after incubation in pepsin and after subsequent incubation in pancreatin.

Author Contributions: L.G.: methodology, formal analysis, investigation, validation, writing—original draft. J.K.: formal analysis, investigation. W.W.: formal analysis, investigation. M.R.: formal analysis, investigation. T.G.: formal analysis, investigation. P.W.: formal analysis, investigation. M.H.: conceptualization, methodology, formal analysis, investigation, supervision, project administration, writing—original draft, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The whole blood used for this investigation was obtained from anonymized volunteers. This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Academic Integrity and Ethics Committee of the Danube Private University (DPU-EK/010).

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: All data and detailed protocols are freely available upon request to the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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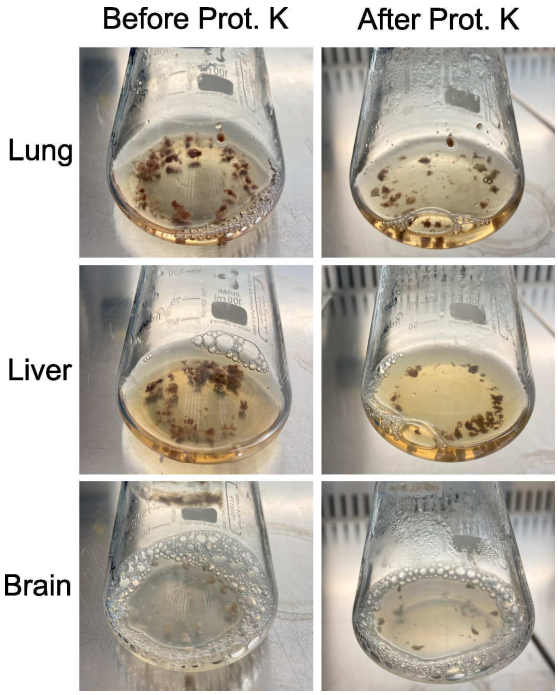

Figure S1. Enzymatic digestion of porcine lung, liver and brain samples using proteinase K (Prot. K). The images depict homogenized lung, liver and brain tissue in the digestion solution before and after incubation in proteinase K, as microscopic examination was not feasible.

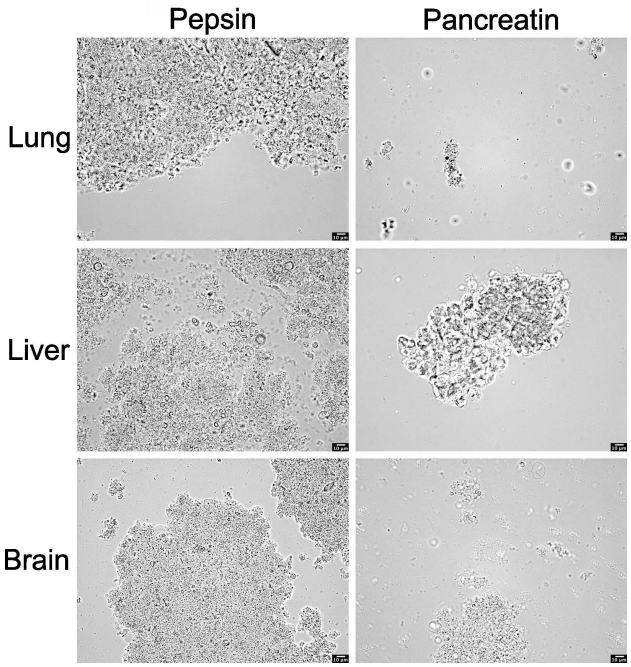

Figure S2. Enzymatic digestion of lung, liver and brain tissue using pepsin and pancreatin. The kidney homogenates were incubated for 4 h in a 2 mg/mL pepsin solution, followed by an additional 24.5 h in a 2 mg/mL pancreatin solution at 37 °C. Representative microscopic images show the progress of digestion after incubation in pepsin and after subsequent incubation in pancreatin.

2.3 Effects of micro- and nanoplastics on blood cells in vitro and cardiovascular parameters in vivo, considering their presence in the human bloodstream and potential impact on blood pressure

Geppner, L., Hellner, J., & Henjakovic, M. (2025). Effects of Micro- and Nanoplastics on Blood Cells In Vitro and Cardiovascular Parameters In Vivo, Considering Their Presence in the Human Bloodstream and Potential Impact on Blood Pressure. Environmental Research, 121254. https://doi.org/10.1016/j.envres.2025.121254

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2.3.1 Contribution

CRediT authorship contribution statement

Liesa Geppner: Writing – original draft, Investigation. Julius Hellner: Writing – original draft, Investigation. Maja Henjakovic: Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Conceptualization.

Indication of own contribution

In the following review publication, my part involved revising and expanding the literature pre-selected by the co-first author Julius Hellner by incorporating newly identified, recent studies to ensure a comprehensive and highly contemporary analysis. My contributions included systematically classifying the literature and organizing the results into structured tables to provide a more comprehensive overview of existing research. Additionally, I collaborated with Maja Henjakovic to establish the link between MPs, NPs, and blood pressure regulation in the discussion section. Furthermore, my role included assisting in editing the editor's and reviewers' comments during the revision and resubmission process.

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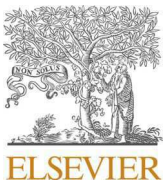

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Review article

Effects of micro- and nanoplastics on blood cells in vitro and cardiovascular parameters in vivo, considering their presence in the human bloodstream and potential impact on blood pressure

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ABSTRACT

The adverse effects of plastics on the environment, wildlife, and human health have been extensively studied, yet their production remains unavoidable due to the lack of viable alternatives. Environmental fragmentation of larger plastic particles generates microplastics (MPs, 0.1–5000 μm) and nanoplastics (NPs, 1–100 nm), which can enter the bloodstream through inhalation or ingestion.

This review examines whether MPs and NPs influence blood pressure. To address this question, relevant studies were analyzed based on predefined criteria.

Due to anatomical barriers and microcirculatory dynamics, only NPs and small MPs are expected to enter the bloodstream under physiological conditions, although pathological states may alter this. In vitro research indicates that MPs and NPs negatively affect erythrocytes and endothelial cells, while rodent models suggest potential cardiovascular effects. Plastic particles and fibers have been detected in human blood, thrombi, atherosclerotic plaques, and various tissues. However, validated data on plastic particle-related blood pressure changes remain lacking. Despite limitations in their applicability to human physiology, preclinical models suggest that MPs and NPs circulate in the bloodstream, interact with blood cells, and contribute to vascular damage. Mechanisms such as endothelial injury, platelet activation, inflammation, and MPs/NPs accumulation in atherosclerotic plaques may contribute to blood pressure elevation but are unlikely to be the exclusive cause of hypertension.

Further research is needed to clarify the role of plastic particles in blood pressure regulation. Standardized detection methods, real-world scenario-related models, and targeted human studies are essential to assessing cardiovascular risks associated with MP and NP exposure.

1. Introduction

Plastics have a wide range of applications, favorable raw materials, good formability, and long durability, which collectively make them difficult to replace. It is therefore evident that conventional plastics are present in almost all areas of human life. The elimination of plastics from everyday life will necessitate a significant investment of time and resources in research, as well as political and financial concessions from society. There are numerous types of plastic, with the most prevalent polymers being polyethylene (PE), polypropylene (PP), poly(ethylene terephthalate) (PET), poly(vinyl chloride) (PVC), and polystyrene (PS) (Santos et al., 2023).

The weathering, aging, and mechanical abrasion of building materials, medical equipment, food packaging, and other plastic objects have been observed to result in the release of small plastic particles, which are now suspected of being harmful to human health. The numerous scientific findings of plastic contamination in water, soil and air, coupled with the indispensable role of conventional plastics, highlight the urgent need to address the current lack of solubility of this problem and the alarming rate of microplastics (MPs) and nanoplastics (NPs) contamination (Athulya et al., 2024; Chen et al., 2024; Giannattasio et al., 2024).

Primary MPs and NPs are intentionally manufactured particles used in various industrial and consumer applications. Secondary MPs and NPs result from the fragmentation of larger plastic pieces. The size of MPs

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ranges from 0.1 to 5000 μm, while NPs range from 0.001 to 0.1 μm (1–100 nm) (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2016).

In recent years, the presence of plastic particles has been identified in a range of human tissues, organs and body fluids. The initial detections revealed the presence of plastic particles in intestinal excretions, substantiating the hypothesis that humans ingest and absorb plastic particles on a daily basis, and that these particles accumulate in the intestine (Schwabl et al., 2019). Subsequently, the presence of plastic particles was also identified in intestinal tissue, thereby corroborating this pathway of plastic uptake (Ibrahim et al., 2021). It has also been demonstrated that MPs can be taken up by inhalation of ambient air and that they are present in human lung tissue (Amato-Lourenço et al., 2021). Furthermore, MPs were identified in human placenta and liver samples (Braun et al., 2021; Horvatits et al., 2022; Ragusa et al., 2021). In early 2022, the first detection of plastic particles larger than 0.7 μm in human blood samples was published (Leslie et al., 2022). Other studies have identified the presence of MPs in a range of other human body fluids, including breast milk, saliva, urine, seminal fluid and tissues (Huang et al., 2022; Li et al., 2024; Montano et al., 2023; Pironti et al., 2022; Ragusa et al., 2022; Weingrill et al., 2023; Zhao et al., 2023).

In animal studies, artificially produced MPs and NPs have frequently been administered orally and inhaled, subsequently demonstrating the distribution and accumulation of particles in various organs (Hu et al., 2021; Kim et al., 2021; Meng et al., 2022; Rawle et al., 2022; Schwarzfischer et al., 2022; Shan et al., 2022).

The findings on MPs in humans, along with numerous animal studies to date, demonstrate that NPs and small MPs can be ingested or inhaled, traverse cellular barriers in the lungs and intestines, enter the bloodstream, and become trapped and accumulate in organs. This review of the literature led us to the question of whether and to what extent plastic particles in the bloodstream can influence blood pressure in humans. Due to anatomical structures and microcirculatory dynamics, it is expected that only NPs and small MPs up to about 5–10 μm will circulate in the blood, while larger particles are likely to be deposited in capillary vessels.

It is hypothesized that the deposition of these small plastic particles in vessels and organs increases total peripheral resistance, thereby increasing blood pressure. It is also thought that the interaction of MPs and NPs with erythrocytes can lead to their damage and with platelets to their activation, which can lead to changes in blood flow. In addition, plastic particles can cause numerous small injuries to the endothelium, which may ultimately lead to a narrowing of the vessel lumen, potentially resulting in changes in blood pressure.

The impact of ingested plastic particles on blood pressure remains uncertain. This review aims to consolidate existing knowledge on this topic and facilitate future studies aimed at elucidating the underlying mechanisms.

2. Methods of literature research

The present literature review was compiled from scientific articles published between December 2011 and July 2024, focusing on the effects and detection of MPs, NPs and airborne bone particles in in vitro and in vivo animal and human studies. Search resources included PubMed (https://pubmed-ncbi-nlm-nih-gov), Science Direct (https://www.sciencedirect.com) and Google Scholar (https://scholar.google.at). The main search terms were microplastics, nanoplastics, cardiovascular disease and hypertension. For the more specific chapters, we included the following search terms: erythrocytes, red blood cells, endothelium, human blood, human heart, cardiotoxicity, air pollution.

The articles were selected based on the criteria of having undergone peer review and written in English. Studies included in this review explored the effects of MPs and NPs on cardiovascular parameters, which consequently influence blood pressure. Accordingly, studies investigating the molecular mechanisms of toxicity, relevant in vitro models, in vivo studies, and human clinical trials were included. Studies were excluded if they focused solely on the ecotoxicological or environmental aspects of MPs without relevance to human health. Additionally, research on macroplastics or other pollutants unrelated to MPs and NPs was not considered. Studies that did not specifically investigate cardiovascular effects or utilized models that are not comparable to human biological systems were also excluded (Table 1).

However, it is also important to recognize the limitations and biases of the included references. A significant proportion of the included publications employed in vitro or in vivo models, which, despite their relevance, may not fully replicate human physiological responses. Additionally, variations in experimental design, such as differences in particle size, concentration, and exposure duration, may compromise the comparability of findings across studies. It is also crucial to recognize that studies reporting null or negative results may be underrepresented due to publication bias.

3. Results

3.1. Pathways of plastic particles into the bloodstream

3.1.1. Intestinal uptake of MPs and NPs

The ingestion of plastic particles and fibers through the digestive tract is unavoidable due to the widespread use of plastic packaging for food and beverages, as well as the presence of plastic particles in food and ambient air that settle on our meals (Mamun et al., 2023; Qian et al., 2024; Xu et al., 2025). To enter the bloodstream from the intestinal lumen, these particles must traverse several biological barriers.

The intestinal mucus layer serves as the first line of defense. Particles larger than 500 nm have been shown to be unable to pass through, as the average mesh size of mucus is around 214 nm (Van Wijngaarden et al., 2025). Surface charge also plays a crucial role in diffusion: particles with a neutral or slightly negative charge exhibit the highest diffusivity (Van Wijngaarden et al., 2025).

Enterocytes are capable of taking up and transporting particles, at least those in a size range of 0.1–1 μm, via several pathways, including caveolae-mediated transcytosis, macropinocytosis, clathrin-mediated transcytotic pinocytosis, and the chylomicron pathway (Delon et al., 2022; Sheth et al., 2021). The resulting vesicles can vary in size from 50 to 2500 nm (Delon et al., 2022; Sheth et al., 2021).

M cells, specialized epithelial cells within the follicle-associated epithelium of Peyer's patches, efficiently uptake particles ranging from approximately 1 to 5 μm via transcytosis and deliver them to underlying immune cells (Delon et al., 2022; Ermak, 1998).

Paracellular passage is generally restricted to particles smaller than 50 nm. However, when tight junction integrity is compromised, the

Table 1
Inclusion and exclusion criteria for MPs and NPs research.

Selection criteria	Inclusion criteria	Exclusion criteria
Content variables	Micro- and nanoplastic particles and fibers	All other types of particles
	Mechanical influence of MPs and NPs on the blood circulation system	Release and uptake of MPs and NPs and the influence on all other organ systems
	Studies on humans, human cells or animal model organisms with a comparable blood circulation	Animals with far comparable blood circulation systems (e.g. fish)
	Diseases caused by MPs and NPs affecting blood pressure	Diseases with no confirmed effect on blood pressure caused by MPs and NPs
Study design	Original research, meta-analyses, pilot studies, studies investigating mechanistic approaches	Reviews
Period of time	2011–2024	
Language	English	All other languages

intercellular space can expand, allowing particles as large as 2 μm to pass through due to increased permeability and reduced barrier resistance (Delon et al., 2022). Temporary gaps caused by the detachment of epithelial cells at the villus tips can also facilitate the passage of larger microparticles (7–70 μm) across the intestinal barrier (Delon et al., 2022). This loss of barrier function is often observed in the context of inflammatory processes (Agrawal et al., 2024). Alternative routes of entry include uptake by macrophages or dendritic cells, followed by translocation through the intercellular spaces of the epithelium into the lamina propria, where particles enter the lymphatic vessels (Prata, 2023). Based on typical cell sizes, it is unlikely that particles larger than 10 μm will be taken up by immune cells for transport.

It can be assumed that plastic particles with a predominantly hydrophobic surface can enter the lymphatic capillaries, which have an average diameter of 10–75 μm, and follow a transport route in the bloodstream similar to that of chylomicrons. The lymph then flows through the thoracic duct into the subclavian vein, from where it enters the right heart and lungs (Geiger, 2024). From the lungs, the particles must pass through the alveolar capillary network, which has a diameter of only 5–10 μm (Grotberg and Romanò, 2023).

For plastic particles with a predominantly hydrophilic surface and a diameter of 5–10 μm, the entry route via the capillary blood network of the intestinal wall should be more likely than via the lymph. This route leads via the vena portae to the liver, where the particles enter the secondary capillary system of the hepatic sinusoids. The sinusoids form a complex network that acts as an immediate filter for larger particles (Burt et al., 2022).

For example, in the case of intestinal inflammation, the activity of immune cells and the permeability of capillaries or other vessels to plastic particles may increase, likely leading to a higher uptake of MPs and NPs.

3.1.2. Pulmonary uptake of MPs and NPs

Under physiological conditions, larger plastic particles are trapped in the upper airways, with only particles smaller than 5 μm reaching the lower airways (Joshi et al., 2025; Kelly and Fussell, 2012). However, depending on exposure intensity, oxidative stress, and inflammatory processes, even larger plastic fragments can enter the lower airways, as demonstrated in independent studies (Amato-Lourenço et al., 2021; C. Chen et al., 2023; Jenner et al., 2022; Momeni et al., 2025; S. Wang et al., 2023). The air-blood barrier is least pronounced in the alveoli, making the infiltration of plastic particles and fibers most likely in this region (Patel et al., 2024).

Similar to the intestinal system, plastic fragments with a maximum diameter of 5–10 μm can enter the pulmonary capillaries directly after damage to the alveolar epithelial cells. Alternatively, particles may be taken up by macrophages from the alveoli and transport via the lymphatic system from the interstitial space into the venous system is possible, allowing them to re-enter pulmonary perfusion (Geiser, 2002; Mori et al., 2013; Warheit and Hartsky, 1993). Particles and fibers larger than 10 μm can also migrate from the lung interstitium via the lymphatic vessels into the venous system and subsequently re-enter the pulmonary capillaries. It is assumed that these mechanisms of particle entry into the blood vessels of the human lung may be altered by oxidative stress, toxic or inflammatory influences, and various pulmonary diseases.

3.2. Effects of MPs and NPs in vitro

3.2.1. Effect of MPs and NPs on erythrocytes

Once MPs and NPs are in the circulation, they can interact with blood and the contained cellular components, such as erythrocytes or thrombocytes (Fig. 1, Table 2) (Christodoulides et al., 2023; Gopinath et al., 2019; Kim et al., 2022; Zhu et al., 2023). The assumption of these interactions led to the investigation of aggregation and adhesion of erythrocytes after treatment with 50, 107 and 250 nm PS MPs and NPs in concentrations of 50, 150, 250, 350 and 500 μg/mL in protein free medium (Barshtein et al., 2011, 2016). Significant effects for hemolysis of erythrocytes were observed only for 50 nm PS NPs, but at all concentrations mentioned above (Barshtein et al., 2011). Additionally, formation of erythrocytes aggregates was observed for 250 nm PS and elevated RBC adhesion to endothelial cells were observed for 107 and 250 nm PS, both only at a concentration of 500 μg/mL (Barshtein et al., 2016). Notably, the experiments by Barshtein and colleagues were carried out in a protein-free medium, while plasma proteins can modulate the properties of NPs, and subsequently their interaction with cells (Cedervall et al., 2007). A study of Gopinath and colleagues analyzed the interaction of proteins with NPs in a size of 100 nm and at a concentration range from 1 to 10 μg/mL and revealed that coronated NPs examined at concentrations between 2.5 μg/mL and 10 μg/mL resulted in cytotoxicity rates between 40 % and 70 %, while hemolysis ranges from 50 % to 90 % at concentrations between 5 and 10 μg/mL (Gopinath et al., 2019). Toxic activity was also observed when testing 100 nm PS-NH_2 NPs, inducing hemolysis of erythrocytes. This activation of secondary hemostasis involves clotting factors initiating the polymerization of fibrin, which stabilizes the platelet plug and entraps the erythrocytes in the fibrin network to form a red thrombus. But

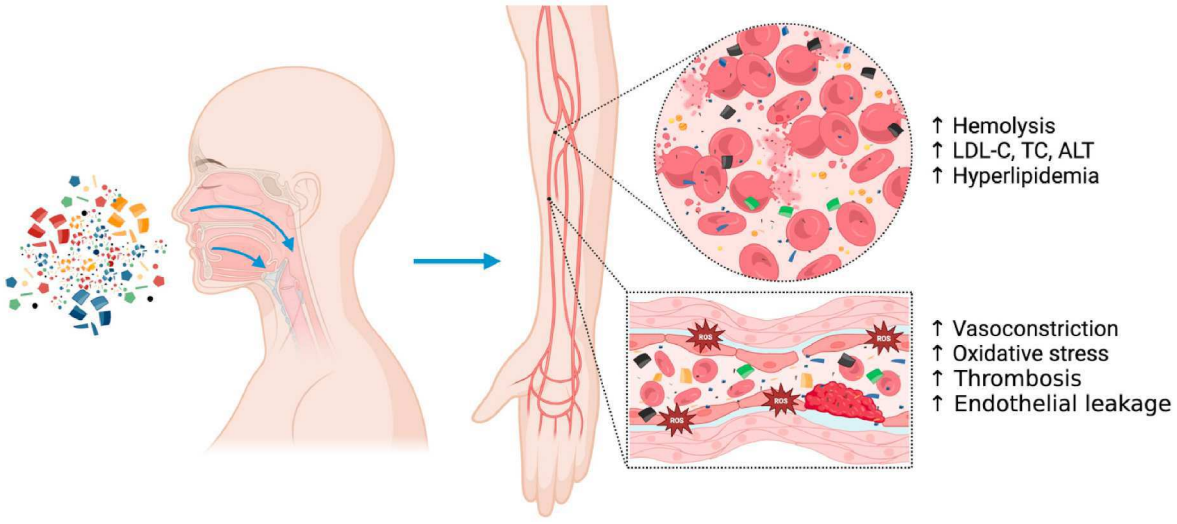

Fig. 1. The hypothesized model of MPs and NPs interactions with human erythrocytes and endothelial cells under high-exposure conditions in vivo. This model illustrates potential consequences such as hemolysis, oxidative stress, endothelial damage, and thrombus formation.

Table 2
Summary of micro- and nanoplastic toxicity in vitro.

Models	Particle properties that caused effects	Concentrations that caused effects	Effect	Investigated type and size of particles	Investigated concentration	References
Human erythrocytes	50 nm PS	≥50 μg/mL	Hemolysis	50, 107 and 250 nm PS	50, 150, 250, 350 and 500 μg/mL	Barshtein et al. (2011)
	250 nm PS	500 μg/mL	RBCs aggregation	50, 107 and 250 nm PS	50, 200 and 500 μg/mL	Barshtein et al. (2016)
	107 and 250 nm PS	500 μg/mL	RBCs adhesion to endothelial cells			
	100 nm PS	≥5 μg/mL	Hemolysis	100 nm PS	1, 2.5, 5, 7.5, 10 μg/mL	Gopinath et al. (2019)
	100 nm PS	≥2.5 μg/mL	Cytotoxicity			
	100 nm PS-NH_2	50 and 100 μg/mL	Hemolysis	100 nm PS-NH_2	10, 25, 50 and 100 μg/mL	Kim et al. (2022)
	100 nm PS-NH_2	50 and 100 μg/mL	RBCs adhesion to HUVECs			
	10 μm PE	≥100 μg/mL	Bilayer tension	0.8 μm PS	25, 100, 200, 300, 400 and 500 μg/mL	Fleury and Baulin (2021)
	8 μm PMMA			1 and 10 μm PE		
				1 and 8 μm PMMA		
	29, 44, 72 nm PS	100 and 200 μg/mL	Hemolysis	29, 44, 72 nm PS	0.001, 0.01, 0.1, 1, 10, 25, 50, 100 and 200 μg/mL	Płuciennik et al. (2023)
	100 nm and 1 μm PS	5, 10, 50 and 100 μg/mL	Hemolysis	100 nm and 1 μm PS	0.1, 1, 5, 10, 50 and 100 μg/mL	Remigante et al. (2024)
	100 nm and 1 μm PS	1 μg/mL	ROS accumulation	100 nm and 1 μm PS	1 μg/mL	
Sheep erythrocytes	460 nm and 1 μm PS	100 μg/mL	Hemolysis	460 nm, 1, 3, 10, 40 and 100 μm PS	1, 10, 100, 500, and 1000 μg/mL	Hwang et al. (2020)
Human umbilical vein endothelial cells (HUVECs)	50 nm PMMA-COOH	50 and 500 μg/mL	Endothelial leakiness	30 nm PS-NH_2	50 and 500 μg/mL	Wei et al. (2022)
	30 nm PS-NH_2	50 and 500 μg/mL	Reduction of cell viability	50 nm PMMA-COOH		
	50 nm PS-NH_2	≥5 μg/mL	Reduction of cell viability	50 nm PS and PS-NH_2	5, 10, 15, 20, and 25 μg/mL	Fu et al. (2022)
	50 nm PS-NH_2	20 μg/mL	ROS accumulation			
	50 nm PS	10 and 20 μg/mL				
	1 μm PS	100 μg/mL	Reduction of cell viability	1 μm PS	5, 10, 25, 50, and 100 μg/mL	Lu et al. (2023)
	500 nm PS	50 and 100 μg/mL	Reduction of cell viability	100 and 500 nm PS	5, 10, 25, 50 and 100 μg/mL	Lu et al. (2022)
	20 nm PS	≥50 μg/mL	Reduction of cell viability	20, 50 and 100 nm PS	50, 250 and 1000 μg/mL	Zhang et al. (2022)
	50 nm PS	250 and 1000 μg/mL				
	100 nm PS	1000 μg/mL				
	20, 50, 100, 500 nm PS	1000 μg/mL	ROS accumulation	20, 50, 100, 500 nm PS	1000 μg/mL	
	5 and 10 μm PS			5 and 10 μm PS		
	0.5 μm PS	≥20 μg/mL	Reduction of cell viability	0.5, 1 and 5 μm PS	20, 40, 60, 80 and 100 μg/mL	Lee et al. (2021)
	1 μm PS	80 and 100 μg/mL				
Human umbilical vein endothelial cell line (EA.hy926)	2.2–6.5 μm PS	≥4×10^{-2} μg/mL	Reduction of cell viability	2.2–6.5 μm PS	4×10^{-6}, 4×10^{-4} 4×10^{-2}, 4 and 40 μg/mL	Chen et al. (2023)
		4×10^{-6} and 4×10^{-2} μg/mL	ROS accumulation			
Murine myocardial endothelial cells	1 μm PS	5.4×10^{-4} μg/mL	Endothelial activation, monocyte cell adhesion	1 μm PS	5.4×10^{-4}, 5.4×10^{-2}, and 5.4 μg/mL	Vlacil et al. (2021)
Porcine coronary artery endothelial cells (PCAECs)	22 nm PS	1 and 10 μg/mL	Endothelial senescence and dysfunction	22 nm PS	1 and 10 μg/mL	Dhakal et al. (2023)
	25 nm PS	1 and 10 μg/mL	Endothelial senescence and dysfunction	25 nm PS	0.1, 1 and 10 μg/mL	Shiwakoti et al. (2022)
Porcine aortic endothelial cell line (AOC)	100 nm PS	5, 25 and 75 μg/mL	Increase in metabolic activity, and VEGF production	100 nm PS	5, 25 and 75 μg/mL	Basini et al. (2023)

erythrocytes can also adhere directly to the endothelium, contributing to the stabilization of the thrombus. This adhesion occurs independently of the hemolytic processes and serves as an additional mechanism to secure the clot to the vascular wall. Adhesion to primary human umbilical vein endothelial cells (HUVECs) was observed at a concentration of 50 and 100 μg/mL, with an extent of hemolysis of 26 % at 100 μg/mL, while no hemolytic effects were detected for plain 100 nm PS NPs at 100 μg/mL (Kim et al., 2022). In contrast, the application of small MPs resulted in the induction of hemolytic effects on erythrocytes (Hwang et al., 2020). It was shown that PS microspheres with diameters of 460 nm and 1 μm affected erythrocytes and showed hemolysis of erythrocytes at a concentration of 100 μg/mL, however PS particles with diameter of 3, 10, 40 and 100 μm were not significantly cytotoxic (Hwang et al., 2020). Furthermore, it was investigated, if MPs in various sizes and types (0.8 μm PS, 1 μm and 10 μm PE, and 1 μm and 8 μm PMMA) and different concentrations (25–500 μg/mL) can destabilize lipid membranes in erythrocytes by mechanical stretching (Fleury and Baulin, 2021). The results showed that no significant conductive signals could be measured on a bilayer in the presence of MPs, but an increase in bilayer tension with increasing concentration was observed, especially for 8 μm PMMA and 10 μm PE particles (Fleury and Baulin, 2021). Płuciennik et al. demonstrated that the in vitro hemolysis of human erythrocytes induced by plain PS NPs, with sizes of 29 nm, 44 nm, and 72 nm, and at concentrations ranging from 0.001 to 200 μg/mL, is dependent on nanoparticle size (Płuciennik et al., 2023). It was shown, that the smallest nanoparticles (29 nm) caused the greatest hemolysis, while larger particles (72 nm) with a higher absolute negative zeta potential (−42 mV) exhibited a lower cytotoxic effect compared to smaller particles with a lower zeta potential (−29.68 mV), with significant effects observed across all particle sizes at concentrations of 100 and 200 μg/mL (Płuciennik et al., 2023). Remigante and colleagues investigated the hemolysis of erythrocytes following treatment with 100 nm and 1 μm PS particles at concentrations of 0.1, 1, 5, 10, 50 and 100 μg/mL, observing significant hemolysis for both particle sizes at even at concentrations of 5 μg/mL and higher after exposure durations of 3 h and 24 h (Remigante et al., 2024). Additionally intracellular reactive oxygen species (ROS) levels were measured in human erythrocytes treated with 1 μg/mL of 100 nm and 1 μm PS particles, revealing significant ROS accumulation

for both particle sizes as early as 30 min post-treatment (Remigante et al., 2024).

3.2.2. Effects of MPs and NPs on the endothelium

The MPs and NPs that have entered the bloodstream circulate in the bloodstream and can also interact with the endothelial cells. Therefore, numerous studies have investigated the effects of plastic particles on endothelial cells (Fig. 1, Table 2).

Specifically, PMMA NPs with a size of 50 nm can induce statistically significant endothelial leakiness at both 50 and 500 μg/mL when investigated on HUVECs, while, in contrast, no endothelial leakiness was observed with the treatment of 30 nm PS-NH_2 NPs (Wei et al., 2022). In terms of cell viability, the opposite is the case: PS-NH_2 shows statistically significant reduction of cell viability at a concentration of 50 and 500 μg/mL, while PMMA has better biocompatibility and no significant reduction of cell viability (Wei et al., 2022).

Similar results, regarding significance for the reduction of cell viability, were also observed in HUVECs with 50 nm a PS-NH_2 NPs at exposure concentrations of 5, 10, 15, 20, and 25 μg/mL, after 12 h of incubation (Fu et al., 2022). While these positive charged PS NPs show high toxicity, the plain 50 nm PS NPs showed no concentration-dependent cytotoxicity (Fu et al., 2022). For the effect of ROS production, the PS-NH_2 NPs show statistical peak at 20 μg/mL, while plain PS NPs revealed significantly high ROS level even at 10 μg/mL (Fu et al., 2022). This reduction in cell viability, along with the impaired mitochondrial membrane potential and increased ROS generation, underscores the cytotoxic effects of NPs. Cytotoxicity on HUVECs was also investigated for 100 nm and 500 nm (Lu et al., 2022) and 1 μm PS particles (Lu et al., 2023) in a wide concentration range between 5 and 100 μg/mL. However, only after 48 h of exposure, the 500 nm PS particles at a concentration of 50 and 100 μg/mL and the 1 μm PS particles at a concentration of 100 μg/mL showed cytotoxicity, while no toxicity associated with oxidative stress was found for any particle size at any concentration (Lu et al., 2022, 2023). Further cell viability tests on NPs with a size of 20, 50, and 100 nm were tested at 50, 250 and 1000 μg/mL on HUVECs and revealed significant effects at 1000 μg/mL for all sizes (Zhang et al., 2022). At 250 and 50 μg/mL, the 100 nm particles showed no effects, and the 50 nm particles exhibited effects only at 250 μg/mL. Notably, the 20 nm particles displayed statistically significant effects at all tested concentrations (Zhang et al., 2022). The investigation of ROS production showed statistically significant increases for NPs and MPs in a size range between 20 nm and 10 μm at a concentration of 1000 μg/mL and with a higher amount of ROS for smaller plastic sizes (Zhang et al., 2022). Regarding reduced cell viability, another study on PS MPs showed a dose- and size dependent inhibition of endothelial cell viability in HUVECs after 72 h of incubation, with significant reduction for 500 nm at all tested concentrations between 20 and 100 μg/mL, while 1 μm PS showed significant results only at 80 and 100 μg/mL and the 5 μm PS exhibited no significant reduction of cell viability at any concentration (Lee et al., 2021).

Furthermore, in a human umbilical vein endothelial cell line (EA. hy926 cells), an average particle size of 4 μm PS MPs have been shown to significantly decrease cell viability after 24 h and at exposure concentrations of 0.04 μg/mL, 4 μg/mL, and 40 μg/mL (Chen et al., 2023). Additionally, a significant accumulation of ROS was observed at both 4 $\times 10^{-6}$ and 0.04 μg/mL (Chen et al., 2023).

Further in vitro studies investigated interactions of 1 μm PS MPs in a concentration of 5.4 $\times 10^{-4}$, 5.4 $\times 10^{-2}$ and 5.4 μg/mL in a murine myocardial endothelial cell line (MyEND) and identified no cell viability, but endothelial activation and monocyte adhesion was observed for the lowest concentration (Vlacil et al., 2021). Another study using primary porcine coronary artery endothelial cells (PCAECs) found, that 22 and 25 nm PS NPs, in a concentration of 1 and 10 μg/mL, induce endothelial senescence and dysfunction (Dhakal et al., 2023; Shiwakoti et al., 2022). Basini et al. studied the interaction between the porcine aortic endothelial cell line (AOC) and 100 nm PS NPs and found a significant increase in metabolic activity and vascular endothelial growth factor (VEGF) at a concentration of 5, 25 and 75 μg/mL (Basini et al., 2023).

3.3. Effects of MPs and NPs in vivo animal models

Moreover, studies have explored the in vivo effects of MP and NP exposure, along with the potential cardiovascular toxicities (Table 3). Exposure of 5 μm or 10 μm PS MPs in a concentration of 1000 μg/L in drinking water to C57BL/6 mice resulted in different cardiovascular toxicities, like vascular histopathological alterations (Shi et al., 2022), reduced cardiac sodium–potassium pump (Na + -K + -ATPase) activity and N6-methyladenosine (m6A) modification of noncoding RNAs (ncRNAs), which is involved in MP-induced myocardial damage (Zhang et al., 2023) and increased obesity in mice treated with 0.5 and 5 μm PS at a concentration of 1 μg/mL, which is associated with cardiometabolic disease phenotype (Zhao et al., 2022). In addition, a significant increase in eosinophils and basophilic leucocytes was observed in mice treated with 5 μm PS at a concentration of 0.1 and 1 μg/mL (Zhao et al., 2022). A very recent study observed vessel wall thickening and abnormal lipid metabolism with significant elevation of the serum levels of low-density lipoprotein (LDL-C), triglycerides (TG), total cholesterol (TC), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and significant inhibition of the serum high-density lipoprotein (HDL-C) levels in C57BL/6 mice after exposure to 100 nm PS NPs at a higher concentration of 100 μg/mL in drinking water (Zhang et al., 2024). Another study investigated the effects of 50 nm PS NPs on systolic blood pressure and diastolic blood pressure in $ApoE^{-/-}$ mice by oral gavage (Wang et al., 2023). A significant increase in diastolic blood pressure was observed after 10 weeks of exposure to 25 and 250 mg NPs/kg body weight, while systolic blood pressure only showed a statistically significant increase after 19 weeks and only at 250 mg NPs/kg body weight (Wang et al., 2023). In vivo experiments on BALB/c mouse models were conducted to investigate the vascular system after by caudal vein injection with 80 nm of PS, PS-NH_2 and PS-COOH with concentrations of 5, 10 and 20 mg/kg for the plain PS particles and 0.05, 0.5 and 5 mg/kg for the aminated and carboxylated PS particles (Wang et al., 2023). All different particles in all concentrations showed a statistically significant increase in leukocytes, a decrease in body weight (except PS-NH_2 0.05 mg/kg) and an increase in lymphocyte count (except PS-NH_2 0.05 mg/kg) (Wang et al., 2023).

Moreover, the exposure of 5 μm PS MPs at a concentration of 0.5 mg/L in drinking water induced mild calcification in the hearts and ascending aortas of normal rats due to a highly significant increase in relative calcium levels, but without indication of significances (Yan et al., 2023). Further studies demonstrated the effects of 500 nm PS MPs at a concentration of 0.5, 5 and 50 μg/mL in drinking water, in Wistar rats (Li et al., 2020; Wei et al., 2021). It was found that pyroptosis, a programmed cell death associated with inflammation, is induced by the increased expression of NF-κB and NLRP3 (significant at all three concentrations) and of cleaved GSDMD (significant at 5 and 50 μg/mL) in cardiomyocytes (Wei et al., 2021). Additionally, cardiomyocyte apoptosis, through mitochondrial damage with significant upregulation of Bax (at 5 and 50 μg/mL) and downregulation of Bcl-2 (at 50 mg/mL) and oxidative stress with increased MDA level and decreased CAT, GSH-PX and SOD levels (all statistically significant at 5 and 50 μg/mL), was induced (Li et al., 2020). Even smaller aminated PS particles, in the range of 100 nm, were administered by intravenous injection to Sprague-Dawley rats at doses of 0.25, 0.5 and 1 mg/kg, and also led to significant thrombus formation, even at the lowest dose tested (Kim et al., 2022).

3.4. Detection of MPs in human blood and tissue samples

Considering MPs in the blood and cardiovascular system in more detail, there are various physiological barriers that allow different sizes

Table 3
Summary of micro- and nanoplastic toxicity on animal in vivo studies.

Models	Particle properties that caused effects	Concentrations that caused effects	Effect	Investigated type and size of particles	Investigated concentration	References
C57BL/6 mice	5 μm PS	1000 μg/mL	Hyperlipidemia, oxidative and vascular injury	5 μm PS	1000 μg/mL	Shi et al. (2022)
	10 μm PS	1000 g/L	Cardiac Na + -K + -ATPase inhibition, heart injury	10 μm PS	1000 μg/mL	Zhang et al. (2023)
	0.5 and 5 μm PS	1 μg/mL	Adiposity	0.5 and 5 μm PS	0.1 and 1 μg/mL	Zhao et al. (2022)
	5 μm PS	0.1 and 1 μg/mL	Increased levels of eosinophils and basophils			
	100 nm PS	100 μg/mL	Vessel wall thickening, abnormal lipid metabolism (LDL-C, TG, TC, ALT, AST, HDL-C)	100 nm PS	100 μg/mL	Zhang et al. (2024)
ApoE$^{-/-}$ mice	50 nm PS	250 mg/kg	Increase in systolic blood pressure	50 nm PS	2.5, 25 and 250 mg/kg	Wang et al. (2023)
	50 nm PS	25 and 250 mg/kg	Increase in diastolic blood pressure			
BALB/c mice	80 nm PS, 80 nm PS-NH_2, 80 nm PS-COOH	5, 10 and 20 mg/kg, 0.05, 0.5 and 5 mg/kg, 0.05, 0.5 and 5 mg/kg	Increase in leucocytes	80 nm PS, 80 nm PS-NH_2, 80 nm PS-COOH	5, 10 and 20 mg/kg, 0.05, 0.5 and 5 mg/kg,	Wang et al. (2023)
	80 nm PS, 80 nm PS-NH_2, 80 nm PS-COOH	5, 10 and 20 mg/kg, 0.05, 0.5 and 5 mg/kg, 0.5 and 5 mg/kg	Decrease in body weight, Increase in lymphocytes		0.05, 0.5 and 5 mg/kg	
Sprague-Dawley rats	5 μm PS	0.5 μg/mL	Calcification of heart and aorta	5 μm PS	0.5 μg/mL	Yan et al. (2023)
	100 nm PS	0.25, 0.5, and 1 mg/kg	Thrombus formation	100 nm PS	0.25, 0.5, and 1 mg/kg	Kim et al. (2022)
Wistar rats	500 nm PS	0.5, 5 and 50 μg/mL	Pyroptosis (increased NF-κB and NLRP3)	500 nm PS	0.5, 5 and 50 μg/mL	Wei et al. (2021)
	500 nm PS	5 and 50 μg/mL	Pyroptosis (decreased cleaved GSDMD)			
	500 nm PS	5 and 50 μg/mL	Mitochondrial damage (upregulation of Bax)	500 nm PS	0.5, 5 and 50 μg/mL	Li et al. (2020)
	500 nm PS	50 μg/mL	Mitochondrial damage (downregulation of Bcl-2)			
	500 nm PS	5 and 50 μg/mL	Oxidative stress			

of plastic particles to pass through (Fig. 2, Table 4). Plastic particles, like PET, PE, PS and PMMA have already been found in human blood samples from 22 healthy adult volunteers at a concentration of 1.6 μg/mL and at a size greater than 700 nm by using pyrolysis gas chromatography and mass spectrometry (Pyrolysis-GC/MS) (Leslie et al., 2022). Another study used LDIR chemical imaging system for the analysis of 14 blood samples from 7 patients undergoing cardiac surgery for the presence of MP, before and after surgery (Yang et al., 2023). Plastic particles and fibers were consistently detected in all blood samples, ranging from 20 to 184 μm. Predominantly, PA and PET comprised over 70% of the total

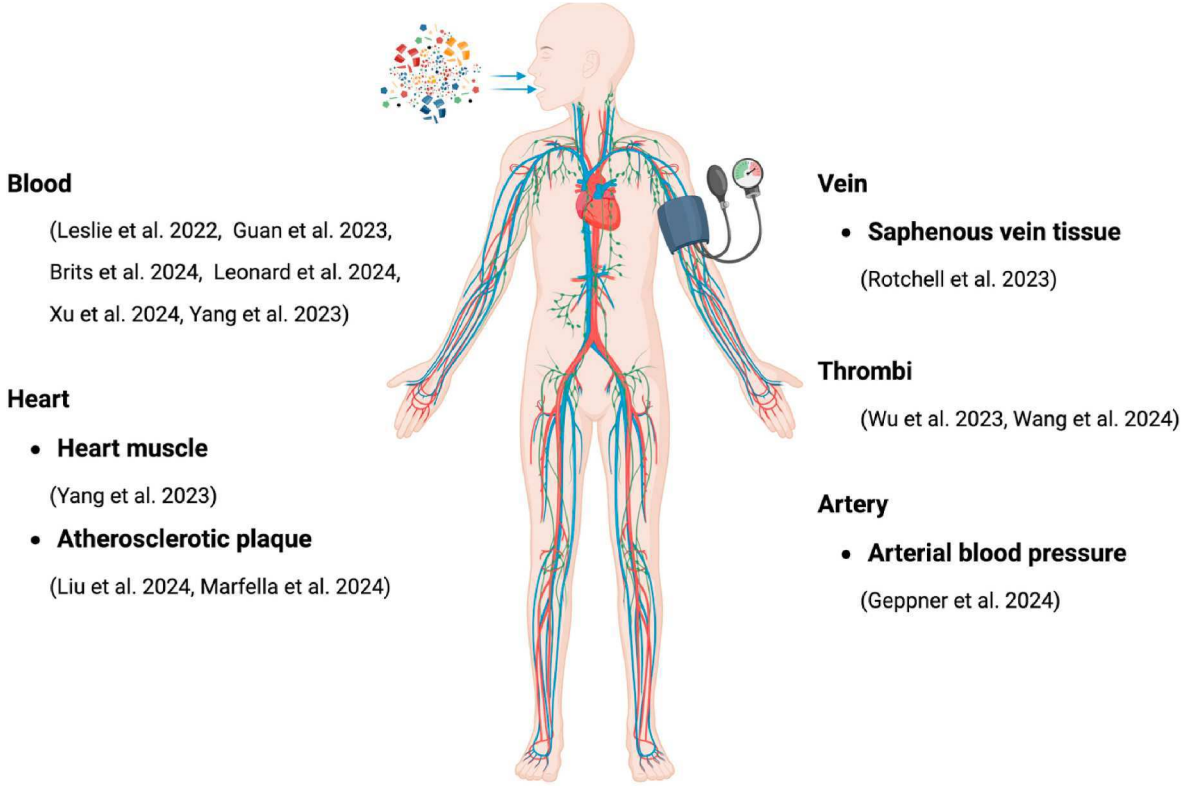

Fig. 2. Ingestion and inhalation of plastic particles and their evidence to date in the human cardiovascular system. Based on the available literature, it can be inferred that plastic particles in the bloodstream may contribute to changes in blood pressure.

Table 4
Summary of detected microplastics in human blood and tissue samples.

Investigated human parameter	Particle properties found	Concentration found	Effect	References
Blood	0.7–500 μm PET > PE > PS > PMMA	1.6 μg/mL	–	Leslie et al. (2022)
	0.3–500 μm PE > PVC > PET > PMMA	1.07 μg/mL	–	Brits et al. (2024)
	19.92–66.73 μm LDPE, PEAA, PSAN and PVA	5 MPs in 8 participants	–	Guan et al. (2023)
	20–184 μm PA and PET (70 %)	–	–	Yang et al. (2023)
	7–3000 μm PE, EPDM, EVA/EVOH, PA and PS (70 %)	–	–	Leonard et al. (2024)
	Ø 20.03 μm PE, PE-co-PP, PP (58 %)	2.2 ± 1.42 MP/mL	–	Xu et al. (2024)
Carotid and coronary artery and aorta	PET, PA-66, PVC and PE	118.66 ± 53.87 μg/g	Atherosclerosis	Liu et al. (2024)
Carotid plaque	<1 μm PE <1 μm PVC	21.7 ± 24.5 μg/mg 5.2 ± 2.4 μg/mg	Risk of infarction and stroke	Marfella et al. (2024)
Left atrial appendage, pericardial and epicardial adipose tissue, myocardium and pericardium	20–469 μm PET, PU (90 %)	–	–	Yang et al. (2023)
Saphenous vein tissue	16–1074 μm Alkyd resin, PVAc, nylon EVA (85 %)	14.9 ± 17.2 MP/g	–	Rotchell et al. (2023)
Arterial thrombi	1–6 μm LDPE	1 piece in 1 out of 26 thrombi	Elevated platelet level	Wu et al. (2023)
Thrombi from patients with ischemic stroke (IS), myocardial infarction (MI), or deep vein thrombosis (DVT)	20–50 μm PE > ACR > PP > CPE > PMMA > PU (94%)	61.75 μg/g in IS thrombi, 141.8 μg/g in MI thrombi, 69.62 μg/g in DVT	Increased disease severity	Wang et al. (2024)
Non-invasive arterial blood pressure measurement	–	–	Increased systolic and diastolic blood pressure	Geppner et al. (2024)

MPs detected, with notable shifts in MPs composition and size observed between pre- and post-surgery blood samples, suggesting potential implications of surgical intervention on MPs dynamics (Yang et al., 2023). The study by Leslie and colleagues was repeated with slight adjustments in a human cohort of 68 whole blood samples, identifying plastic polymers in 64 of the samples (Brits et al., 2024). The most prevalent polymer was PE, followed by PVC, PET and PMMA, with an average total polymer concentration in the blood samples determined to be 1.07 μg/mL and individual concentrations ranging from 0.17 to 2.49 μg/mL (Brits et al., 2024). Guan and colleagues investigated human blood from 8 participants and found two Low density Polyethylene (LDPE) MPs and one MP particle each of Polyethylene-co-acrylic acid (PEAA), Polystyrene-coacrylonitrile (PSAN) and Polyvinyl alcohol (PVA), ranging in size from 19.92 to 66.73 μm, using Raman microspectroscopy (Guan et al., 2023).

Using a similar participant setup, 20 healthy adult volunteers, but with a different methodology, namely micro fourier transform interferometer (μFTIR) spectroscopy, plastic particles were also analyzed from human blood samples (Leonard et al., 2024). The more than 70 % of the detected plastic particles consisted of PE, EPDM (ethylene propylene diene monomer), EVA/EVOH (ethylene-vinyl acetate/ethylene vinyl alcohol), PA, and PS, with a mean particle length of 127.99 ± 293.26 μm (ranging from 7 to 3000 μm) and a mean particle width of 57.88 ± 88.89 μm (ranging from 5 to 800 μm) (Leonard et al., 2024). Another study collected 15 blood samples and detected 2.2 ± 1.42 MP particles per mL, identifying 10 different polymers, with PE, PE-co-PP, and PP being the most common, comprising 23.68 %, 21.05 %, and 13.16 % of the total, respectively, and found that the average length of MPs in blood samples was 20.03 μm, with an average width of 15.93 μm (Xu et al., 2024).

An examination of digested human saphenous vein tissue samples using μFTIR spectroscopy provided the first evidence of MPs contamination in human vascular tissue, detecting microplastics in four out of five vein samples, with an average of 14.9 ± 17.2 MP/g of tissue (Rotchell et al., 2023). The most abundant polymers found were alkyd resin, poly vinyl propionate/acetate (PVAc) and nylon ethylene vinyl alcohol (nylon EVA), with MPs having an average length of 119.59 ± 226.82 μm (range 16–1074 μm) and width of 41.27 ± 62.80 μm (range 7–300 μm) (Rotchell et al., 2023). The presence of MPs in human arterial thrombi was investigated using μRaman spectroscopy, with 16 out of the 26 samples analyzed testing positive for a total of 87 microparticles (Wu et al., 2023). Interestingly, most of the particles were characterized as pigments, in particular, 21 particles were characterized as made by phthalocyanine (predominantly copper phthalocyanine) and only one MP particle, made of low density polyethylene (LDPE), was identified (Wu et al., 2023). According to the authors, the pigments found in thrombi, are added to polymers to provide color, and together with the MP particle this probably originates from daily plastic exposure (Wu et al., 2023). Looking at the association between all the particles found in the thrombi and cardiovascular parameters, the authors found a statistically highly significant positive association between the number of particles in thrombus and blood platelet levels, but no statistical association with systolic blood pressure and diastolic blood pressure (Wu et al., 2023).

Moreover, Pyrolysis-GC/MS and additional stable isotope analysis, and electron microscopy, was used to analyze the carotid plaque tissue of 257 patients and found an average PE concentration of 21.7 ± 24.5 μg/mg plaque in 58.4 % of the samples, of which 12.1 % additionally contained a measurable amount of PVC (5.2 ± 2.4 μg/mg plaque) (Marfella et al., 2024). It was further shown, that patients with plastic particles detected within atherosclerotic plaques exhibited a significantly higher risk of primary endpoint events, including myocardial infarction and stroke, compared to patients without these substances detected (Marfella et al., 2024). Likewise with the help of Pyrolysis-GC/MS MPs (PET, PA-66, PVC, and PE) were detected in various types of human arteries, including coronary and carotid arteries with atherosclerotic plaques, as well as in the aorta without plaques (Liu et al., 2024). Across 17 carotid, coronary or aorta samples, an average plastic concentration of 118.66 ± 53.87 μg/g tissue was detected, with significantly higher concentrations in arteries with atherosclerotic plaques compared to those devoid of such pathological features (Liu et al., 2024). Plastic particles and fibers were also detected in cardiac tissue samples from 15 patients undergoing cardiac surgery using advanced imaging techniques, which identified nine types of MPs with diameters

ranging from 20 to 469 μm across five types of heart tissue, with PET and PU comprising approximately 90 % of the total quantity of MPs (Yang et al., 2023). Another recent investigation detected microplastics, with diameters ranging from 20 to 50 μm, in human thrombi from patients suffering from ischemic stroke, myocardial infarction, and deep vein thrombosis, reporting median concentrations of 61.75 μg/g, 141.80 μg/g, and 69.62 μg/g, respectively, (Wang et al., 2024). Further studies have shown that both microplastics and pigment particles accumulate in thrombi (Wang et al., 2024; Wu et al., 2023). This in turn affects cardiac functions and causes toxicity on vascular sites, which in turn can affect blood pressure (Zhu et al., 2023). This highlights the potential cardiovascular risk posed by foreign particles circulating in the bloodstream.

A further investigation, conducted by our team, investigated the potential effects of a partial plastic diet on blood pressure. The findings of this pilot study indicated a correlation between reduced plastic consumption and lower blood pressure. This observation led to the hypothesis that plastic particles present in the bloodstream may contribute to elevated blood pressure (Geppner et al., 2024).

3.5. Effect of airborne particle inhalation on blood pressure

The impacts of inhaled air particulate matter (PM) on the cardiovascular system have been documented in different animal and human studies (Table 5) and inhalation seems to be the most significant route of human exposure to MPs, with an estimated average daily intake of approximately 127 MPs through ambient air (Cox et al., 2019). A previous study in rat models inhaling polyamide (PA) MPs with a mean diameter of 2.81 μm ($PM_{2.5\text{-}10}$) and a single exposure period of 4 h–9.53 mg/m^3 aerosol showed altered levels of proinflammatory cytokines, with statistically significant elevations in Interleukin-6 levels and trend elevations ($p \leq 0.1$) in C-reactive protein and monocyte chemoattractant protein-1 biomarkers of systemic inflammation (Cary et al., 2023). Additionally, significantly increased mean arterial pressure (MAP) was observed (Cary et al., 2023). Another study examined the effects of particle inhalation in Wistar rats exposed to airborne particulate matter with an aerodynamic diameter of 2.5 μm ($PM_{2.5}$) or less at concentrations of 4 and 40 mg/kg body weight (Wang et al., 2019). It was shown that a significant increase in blood pressure (BP) and heart rate (HR) is only observed at a dose of 40 mg/kg in normal rats, while BP and HR are already statistically significantly increased at 4 mg/kg in hyperlipidemic rats (Wang et al., 2019).

Similar results for BP and HR were also obtained when using ultrafine carbon particles with a median particle size of 31 nm applied by inhalation in a concentration of around 0.18 mg/m^3 in spontaneously hypertensive rats. Blood pressure significantly increased by 4.4 % on the first day and heart rate significantly increased by 6.3 % on the 2nd day of recovery (Upadhyay et al., 2014). Additionally polymorphonuclear neutrophiles significantly increased by 58 %, while lymphocytes remained unchanged and proinflammatory cytokine interleukin 6 was significantly increased by 25 % on the 1st recovery day (Upadhyay et al., 2014). A further study investigated the effects of in utero exposure to ultrafine particles in pregnant C57BL/6J p^{un}/p^{un} mice by intratracheal installation at a ratio of 0.4 mg/kg. They found that this exposure led to significantly increased intrauterine oxidative damage and elevated systolic BP and mean arterial pressure in male offspring (Morales-Rubio et al., 2019).

In human participants, a study investigated, that an exposure to fine particulate pollution ($PM_{2.5}$) by 0.01 mg/m^3 was linked to a heightened risk of acute ischemic coronary events among those with preexisting hypertension, whereas no evidence of increased risk for acute ischemic heart disease was found in participants without seriously diseased coronary arteries (Pope et al., 2006). Another in vivo human study investigated the impact of exposure to concentrated ambient particles with a size of 2.5 μm ($PM_{2.5}$) or less and a concentration of 0.0006 (control), 0.21 and 0.24 mg/m^3, on blood pressure (Bellavia et al., 2013). Compared to the control group, they found that even exposure of only 130 min changes short-term methylation profiles, which is associated with statistic significant elevated systolic BP at a concentration of 0.21 mg/m^3 and high significant elevated systolic BP at a concentration of 0.24 mg/m^3 after exposure, while changes in diastolic BP were not statistically significant (Bellavia et al., 2013).

4. Discussion

The effects of MPs and NPs on blood pressure remain a new area of concern. Numerous studies suggest indirect pathways through which these particles may contribute to cardiovascular changes. This discussion aims to bridge the current evidence and highlight how exposure to MPs and NPs may modulate blood pressure through these underlying biological pathways.

The ability of plastic particles to traverse the cellular barriers of the lungs and intestines allows for their direct release into the capillaries or transport via the lymphatic system to the venous circulation. Their interaction with erythrocyte membranes is unavoidable, potentially resulting in hemolysis and/or alterations in membrane flexibility. Hemolysis, in turn, releases cell-free hemoglobin, which, once it enters the bloodstream, rapidly binds to nitric oxide, a key molecule that promotes vasodilation and regulates blood pressure, resulting in vasoconstriction, increased vascular resistance and ultimately higher blood pressure (Rother et al., 2005). Decreased cell membrane flexibility is known to impede erythrocyte passage through narrow capillaries, leading to their premature clearance in the spleen. Moreover, this phenomenon may increase the likelihood of microembolism formation in diverse tissues, thereby elevating total peripheral resistance within the circulatory system (Kleinbongard and Heusch, 2022).

Furthermore, spherical plastic particles were shown to cause oxidative stress in endothelial cell models by increasing the formation of ROS, which have been implicated in the development of chronic

Table 5
Summary of airborne particle toxicity on blood pressure.

Models	Particle properties that caused effects	Concentrations that caused effects	Effect	Investigated type and size of particles	Investigated concentration	References
Sprague-Dawley rats	2.81 μm PA	9.53 mg/m^3	Systematic inflammation, increased MAP	2.81 μm PA	9.53 mg/m^3	Cary et al. (2023)
Wistar rats (normal) Wistar rats (hyperlipidemic)	≥2.5 μm PM	40 mg/kg 4 and 40 mg/kg	Increased BP and HR	≤2.5 μm PM	4 and 40 mg/kg	Wang et al. (2019)
Spontaneously hypertensive rats	31 nm ultrafine carbon particles	180 $\mu g/m^3$	Increased BP, HR, neutrophiles and IL-6	31 nm ultrafine carbon particles	0.18 mg/m^3	Upadhyay et al. (2014)
C57BL/6J p^{un}/p^{un} mice	Ultrafine particles	0.4 mg/kg	Increased BP and oxidative stress	Ultrafine particles	0.4 mg/kg	Morales-Rubio et al. (2019)
Human	≤2.5 μm PM	0.01 mg/kg	Increased risk of acute ischemic coronary events	≤2.5 μm PM	0.01 mg/kg	Pope et al. (2006)
	≤2.5 μm PM	0.21 and 0.24 $\mu g/m^3$	Increased systolic BP	≤2.5 μm PM	0.21 and 0.24 $\mu g/m^3$	Bellavia et al. (2013)

inflammation, endothelial damage and vascular stiffness, and may ultimately contribute to hypertension (Franco et al., 2022; Shaito et al., 2022). In vivo, the vascular environment is often exposed to more complex and potentially harmful particles, including fibers and sharp-edged fragments, which then could cause inflammatory reactions and plaque formation, especially at sites of high arterial pressure, such as places of vascular branching (Kim, 2023; Patrick et al., 2021). It is well documented that an elevated white blood cell count is associated with the occurrence of hypertension, independent of other traditional cardiovascular risk factors (Ishida et al., 2021; Mansoori et al., 2024; Shankar, 2004).

In addition, in vivo animal studies have shown that exposure to polystyrene MPs and NPs can induce, among other effects, hyperlipidemia and altered lipid metabolism (Shi et al., 2022; Wang et al., 2023; Zhang et al., 2024, 2023; Zhao et al., 2022). It has been suggested that an increase in lipid levels may lead to a subsequent development of hypertension.

Multiple studies have already confirmed the presence of MPs in human blood, with particles of various sizes from 300 nm to several 100 μm and polymer types, highlighting the systemic presence of MPs in the bloodstream (Brits et al., 2024; Leonard et al., 2024; Yang et al., 2023). Consequently, the accumulation of plastic particles in the arteriosclerotic plaques, as evidenced by independent studies (Liu et al., 2024; Marfella et al., 2024), is not unexpected. Plaques can disrupt the laminar blood flow, which can alter hemodynamics and increase peripheral vascular resistance, a factor directly related to blood pressure regulation (Liu and Wu, 2024). The detection of plastics in heart tissue samples and the great saphenous vein (Yang et al., 2023; Rotchell et al., 2023) confirms the systemic presence of plastic particles in the bloodstream and their potential risk of causing vascular damage and obstruction. The high variability in detected particle sizes in human blood and tissue samples, summarized in Tables 4 and is most likely due to differences in isolation and analysis methods. The frequent use of strong chemicals, such as potassium hydroxide (KOH) or hydrogen peroxide (H_2O_2), can induce swelling, clumping, or aggregation of plastic particles (Alfonso et al., 2021; Cole et al., 2014; Geppner et al., 2023). Using commercially available, spherical MPs, these chemically induced changes are easily detectable, but not in realistic plastic fragments and fibers from biological samples. Additionally, infra-red and Raman microspectroscopy often capture only surface signals. Particle aggregation could lead to an apparent increase in particle size, potentially explaining the reported presence of MPs larger than 10 μm in human samples.

The hypothesis that plastic particles in the blood can cause vascular changes and subsequently increase blood pressure is supported by evidence from studies on particulate matter's effects on the cardiovascular system. It has been shown that fine particulate matter, circulating in the human body, can influence systolic and diastolic blood pressure, due to inflammation, oxidative stress and immune responses, which can lead to a change in peripheral vascular resistance (Bartoli et al., 2009; Zhang et al., 2023; Pope et al., 2006; Bellavia et al., 2013). Recent findings reveal that the median daily intake of airborne MPs and NPs was estimated to be 4.62×10^{-6} μg for outdoor environments, while indoor exposure is significantly higher, with a median daily intake of 6.55×10^{-4} μg (Chen et al., 2024). The proportion of plastics in the fine dust fraction depends on prevailing environmental conditions and the specific setting.

In addition to the mechanical effects of plastic particles and fibers on blood pressure, chemical effects must also be considered. Phthalates, often used as plasticizers, can be released from ingested and inhaled MPs and NPs and may also contribute to elevated blood pressure. The most studied populations are those considered to be more vulnerable, i.e. children and pregnant women, but there are also studies in adults showing that various phthalates are associated with increased blood pressure (Han et al., 2020; Yao et al., 2020; Zhang et al., 2018).

5. Conclusion and prospection

In conclusion, the current state of research provides clear evidence that plastic particles can cross the barriers of the lungs and intestines and enter the bloodstream. The uptake of MPs and NPs is significantly increased when these barriers are compromised by damage and associated inflammatory processes. Once in the bloodstream, plastic particles and fibers may damage erythrocytes and endothelial cells, triggering immune responses that restrict blood flow, impair circulation to the heart, and potentially contribute to elevated blood pressure. Research on this topic has predominantly been conducted in cell and animal models, which provide valuable insights but critically do not reflect the human situation. The development of hypertension is a multifactorial condition, and its onset cannot be attributed solely to MPs and NPs. However, available data suggest that these particles may contribute to elevated blood pressure in humans by inducing cardiovascular changes, but validated evidence is still lacking.

Significant research gaps remain that limit our understanding of the impact of plastic particles on blood pressure. Key areas for future investigation include.

- Standardized methods for detecting small MPs and NPs.
- Studies on the effects of MPs and NPs composed of different polymers on cardiovascular parameters in both in vitro and in vivo models, using realistic concentrations, particle sizes, shapes, and polymer mixtures.
- Research linking human blood pressure and other cardiovascular parameters to MPs/NPs levels in the blood, plastic consumption, or specific environmental exposures, while accounting for sex, age, and preexisting conditions.
- Investigations into the potential beneficial effects of reducing plastic intake on hypertension in humans, considering sex, age, and preexisting conditions.

CRediT authorship contribution statement

Liesa Geppner: Writing – original draft, Investigation. **Julius Hellner:** Writing – original draft, Investigation. **Maja Henjakovic:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Conceptualization.

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

No data was used for the research described in the article.

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3 Discussion

The reliable detection and characterization of microplastics in biological samples requires the standardization and validation of simple and non-destructive digestion methods. This doctorial thesis aimed to establish such protocols for both blood and tissue, using enzymatic and chemical approaches that preserve particle integrity. These methodological advances form the basis for assessing the presence of MPs in human samples and exploring their potential contribution to cardiovascular alterations, particularly blood pressure dysregulation. The discussion combines these findings with current evidence on the biological effects of circulating plastic particles.

3.1 Method Development for plastic extraction from human blood

The reliable detection of MPs and NPs in biological samples remains a major challenge, particularly by using digestion methods that lead to potential alteration, deformation and agglutination. Therefore, blood was selected as a suitable matrix as it is an easily accessible and standardized body fluid. The presence of plastic particles in blood indicates that they circulate systemically and can therefore potentially be transported to various organs and tissues, making blood a particularly meaningful matrix, both in methodological and toxicological terms.

Standard chemical digestion techniques, including those that use strong alkalis or H_2O_2, have proved to be effective, even on complex matrices (Ibrahim et al., 2021; Nihart et al., 2025; Ragusa et al., 2021; Rotchell et al., 2023). However, even under mild conditions, these methods often cause degradation of plastics, resulting in decomposition of PS, PC and PET, and damage or discoloration of PE, PA and unplasticized PVC (Al-Azzawi et al., 2020; Cole et al., 2014; Hurley et al., 2018; López-Rosales et al., 2021), which can lead to inaccuracies in subsequent analyses.

To address these limitations, our study evaluated enzymatic digestion methods using proteinase K and pepsin and pancreatin. Although proteinase K demonstrated effective

digestion results, it requires a more complex and time-consuming procedure, which is based on the digestion protocol developed by Leslie and colleagues (Leslie et al., 2022).

In contrast, digestion with pepsin and pancreatin simulates the physiological conditions of the human gastrointestinal tract and reflects the enzymatic environment to which plastic particles are naturally exposed after ingestion (Prabhu et al., 2024). This biologically relevant approach proved to be the most effective for blood digestion, offering gentle matrix degradation while preserving morphology of 5 µm PS particles and enabling reliable downstream identification. Based on these findings, the pepsin and pancreatin method was selected as the most suitable protocol for further analysis of MPs in blood.

However, almost no progress in digestion could be observed for tissue digestion, because compared to blood, mammalian tissues have a higher degree of structural complexity, which requires further methodological adjustments to ensure complete digestion and reliable recovery of plastic particles. This is because proteinase K is only used as a protein denaturing agent to isolate undegraded nucleic acids and does not address other macromolecular components such as lipids and polysaccharides that would be necessary for the digestion of biological tissue (Hilz et al., 1975). Although pepsin can be used to cleave peptide bonds followed by pancreatin which is capable of degrading fats, carbohydrates, and proteins with its contained lipase, amylase, and protease enzymes (Pape et al., 2019), these enzymes also failed to achieve adequate digestion.

3.2 Method optimization for plastic extraction from mammalian tissue

Enzymatic digestion with proteinase K or pepsin and pancreatin has been demonstrated to be a gentle and efficient approach for extracting plastic particles from blood. However, the application of this method to solid biological tissue poses additional challenges. For the present investigations, porcine lung, liver, kidney, and brain tissue was used due to its easy accessibility and good comparability to human tissues. Unfortunately, almost no progress in digestion could be observed for the enzymatic digestion approach.

Alongside the optimization of the pepsin and pancreatin method for tissue digestion, a gentle KOH digestion method was investigated. This method resulted in effective digestion of lung, liver, and kidney tissue, by using 10 % KOH at 37 °C for 76 h, without significant alterations in particle count, size, or surface area of 1 and 5 µm PS particles. However, efficient digestion of brain tissue was not observed. According to the study by Nihart and colleagues, a similar digestion approach was used, with brain tissue treated with 10 % KOH at 40 °C for 3 to 5 days. However, complete digestion was not observed either in this setting (Garcia et al., 2024). The inefficient digestion of brain tissue is probably attributable to its high lipid content. Therefore, future studies should consider incorporating organic solvents, such as methanol or chloroform, to improve the digestion process (Chang et al., 2009; Söderberg et al., 1990). These findings on the non-destructive impact on 1 and 5 µm PS MPs are in accordance with other reports that have validated the applicability of alkaline digestion for soft tissues (Gulizia et al., 2022). However, it is important to note that the digestion properties can be polymer and size specific. Consequently, a general conclusion that ensures non-destructive treatment cannot be drawn solely from the testing of PS particles. Furthermore, it is also necessary to test smaller particles in the nanometer size range, because the smaller the particles the larger the surface area to volume ratio (Abbasi et al., 2023). Therefore, smaller particles could be more susceptible to degradation, which underscores the importance of conducting additional investigations of different polymer properties.

3.3 Effects of ingested and inhaled plastic particles on human blood pressure

The presence of plastic particles in human blood is a cause for concern as it raises critical questions about their potential impact on blood pressure, a parameter that is very sensitive to various environmental factors and serves as an easily quantifiable indicator in cardiovascular studies. This review highlights the fact that while in vitro data and animal studies suggest a possible association between exposure to MP and elevated blood pressure, direct evidence from human studies remains limited. The uncertainty about the physiological effects of

circulating microplastics is further heightened by the current lack of certainty about the validity of reported particle sizes in human samples (Roslan et al., 2024).

The detection of large microplastic particles in various human biological matrices has raised critical questions about their actual presence and the reliability of the detection methods and reported particle sizes. It is unlikely that particles larger than 10 µm are circulating in the vessels, as the capillary network only has a size of 5 to 10 µm. Only via the lymphatic system, which has a diameter of 10 to 75 µm, larger particles can be transported through the body (Geppner et al., 2025). One possible explanation for the occurrence of such large particle sizes could be the use of aggressive chemical digestion methods, which probably alter the physical properties of the MPs and lead to their aggregation. During this process, individual MPs and NPs of different shapes and sizes can aggregate and form larger structures that appear as single particle during analysis (Al-Azzawi et al., 2020). This effect is particularly pronounced for spherical particles, which have been shown to easily aggregate due to the strong surface pressure generated by the corona as they move closer to larger coronated NPs and can coalesce with them (Cole et al., 2014). This finding offers a potential explanation for the presence of large particles measuring up to 3000 µm in blood, up to 500 µm in placental tissue or up to 469 µm in the heart, which were digested with 30 % H_2O_2, 68 % HNO_3 or a mixture of these different chemical reagents, respectively (Leonard et al., 2024; Liu et al., 2023; Yang et al., 2023). However, even in tissues digested with 10 % KOH, particles of significant size were identified. For example, in placental tissue particles measuring up to 307.29 µm were observed, or in testicular tissue particles up to 100 µm were detected using this chemical digestion method (Zhao et al., 2023; Zhu et al., 2023).

In addition to possible particle alteration due to aggressive digestion methods, the use of different microscopic techniques, including variations in illumination settings and optical overlays, may further contribute to misinterpretation of particle dimensions (Fritz et al., 2024). The development of alternative, less aggressive digestion techniques, such as enzymatic methods, could significantly improve the reliability of the data and help avoid size-related artefacts during analysis. This underscores the necessity for a more systematic investigation

into the impact of aggressive, chemical digestion methods particularly on very small plastic particles, and the subsequent standardization of particle identification procedures.

4 Conclusion

This thesis presents key findings from three related studies addressing both methodological and toxicological aspects of microplastic research in human health.

An enzymatic digestion protocol using pepsin and pancreatin, adapted to human physiological conditions, has been successfully established for the isolation of plastic particles from human blood. This method provided gentle matrix degradation while maintaining the integrity of 5 µm polystyrene particles and proved to be less labor-intensive than other enzymatic approaches. In tissue samples, enzymatic digestion with pepsin and pancreatin, showed clear limitations due to the structural complexity of organs. However, parallel investigation of a 10 % potassium hydroxide digestion protocol at physiological temperature allowed efficient digestion of porcine kidney, lung and liver tissue with no detectable changes in the 1 and 5 µm polystyrene particles. Even if both the enzymatic protocol and the chemical digestion method using potassium hydroxide can be considered as a gentle applicable method in relation to particles with a size larger than 1 µm, further investigation of KOH effects on different plastic particle characteristics is necessary.

With regard to the detection of plastic particles in humans, multiple studies have reported the presence and distribution of plastics in human fluids and tissues following oral or inhalative uptake. While evidence from human studies remains limited, existing in vitro and animal data suggest possible mechanisms by which circulating micro- and nanoplastics may contribute to blood pressure changes.

Taken together, the findings of this thesis emphasize the need for standardized, particle-preserving protocols and highlight the relevance of studying exposure to plastic particles in relation to human health. Further interdisciplinary research is essential to refine detection techniques and assess long-term physiological consequences, particularly with regard to blood pressure.

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