

Methods for detecting microplastics in human tissues, with a focus on vascular and intravascular specimens

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Abstract

Due to increasing environmental contamination resulting from human activity, micro- and nanoplastics are becoming increasingly detected within human biological systems. Human exposure occurs primarily through the digestive and respiratory tracts, as well as through dermal contact. In recent years, several studies have identified micro- and nanoplastics not only at these sites of exposure but also within internal human tissues, including the liver, placenta, myocardium, blood, thrombi, and vascular specimens. These findings suggest that such particles may cross biological barriers and disseminate through the circulatory system to distant organs and tissues. At present, however, there is no standardized method for the detection and characterization of micro- and nanoplastics in human tissues. This review summarizes the currently available analytical methods used for the detection of micro- and nanoplastics in human tissues, with particular emphasis on vascular and intravascular specimens, and discusses their applicability, advantages, limitations, and methodological challenges across different biological matrices.

Key words: Raman spectroscopy, microplastics, blood, thrombi, nanoplastics, human tissues, vascular tissues, atherosclerosis.

Introduction

Plastic has become an integral component of both small- and large-scale modern human activity. Synthetic polymers permeate nearly every aspect of daily life, ranging from food packaging and bottled water to medical devices and surgical equipment. Plastics are valued for their durability and resistance to chemical and mechanical degradation; however, these same properties also contribute to their persistence within natural environments. With the continuous increase in global plastic production, combined with the absence of efficient degradation pathways, plastic waste accumulates extensively within ecosystems and increasingly within biological systems as well. Macroplastics, generally defined as plastic particles larger than 5 mm, undergo gradual mechanical, thermal,

chemical, and photodegradative fragmentation within the environment, leading to the formation of progressively smaller particles known as microplastics (MPs). Due to their small size and widespread environmental distribution, MPs are now detectable in aquatic ecosystems, food products, drinking water, and atmospheric particles [1–5]. Consequently, human exposure has become virtually unavoidable.

Human exposure occurs primarily through the gastrointestinal and respiratory tracts, although dermal absorption has also been proposed as a potential route of entry. MPs are broadly categorized into primary and secondary particles [6]. Primary MPs are intentionally manufactured at microscopic dimensions for industrial or commercial purposes, whereas secondary MPs arise from the degradation of larger plastic materials through thermal, hydrolytic, photochemical, oxidative, or biological processes [7]. Both primary and secondary MPs have been identified in a wide range of human tissues and biological specimens, including intestinal tissue [8], lungs [9], kidneys [10], liver [11, 12], spleen, semen [13], breast milk, placenta, fetal meconium [14–16], and bone marrow [17].

Interestingly, several studies suggest that MPs may preferentially accumulate within areas of pre-existing tissue pathology. Increased MP concentrations have been reported in cirrhotic liver tissue compared with healthy liver tissue [12], in prostate cancer and paratumoral specimens [18], and in multiple tumor types, including lung, gastric, colorectal, cervical, and pancreatic malignancies [19]. MPs have also been identified within atherosclerotic plaques and thrombotic specimens [20–22], raising important questions regarding their potential involvement in vascular inflammation, thrombogenesis, endothelial dysfunction, and atheroma formation. These findings further support the importance of investigating vascular and intravascular tissues as critical interfaces in the systemic distribution and biological interaction of MPs.

Several endogenous physiological barriers, including the intestinal epithelium, alveolar epithelium, vascular endothelium, blood-brain barrier, placental barrier, and blood-testis barrier, normally function as selective filters limiting the uptake of larger particles. Nevertheless, smaller MPs and nanoplastics (NPs) may penetrate these barriers under certain conditions, particularly when particle size, surface properties, and local inflammatory processes favor translocation. Experimental studies have demonstrated that murine intestinal mucosa can take up 40 nm NPs through caveolin- and clathrin-mediated endocytosis pathways [23]. Other investigators have shown that nanoparticles may induce endothelial leakiness both *in vitro*

and *in vivo*, thereby increasing vascular permeability and potentially facilitating systemic dissemination [24].

Once particles enter the circulation, they may be distributed rapidly throughout the body. The microvasculature itself may act as a partial mechanical filter, since capillary diameters of approximately 5–8 μm may impede the passage of larger particles and potentially contribute to microcirculatory disturbances [25]. MPs and NPs have subsequently been identified in cerebrospinal fluid, amniotic fluid, semen, urine, and circulating venous blood [25, 26], suggesting that these particles are capable of crossing both healthy and pathologically altered biological barriers. However, the precise mechanisms governing tissue retention, bioaccumulation, redistribution, and excretion remain poorly understood.

Accurate characterization of MPs within biological samples requires the assessment of multiple parameters, including particle size, morphology, polymer composition, surface characteristics, color, and degree of degradation. At present, however, no universally standardized analytical method exists for the identification and characterization of MPs in human tissues. Existing studies remain difficult to compare directly because of differences in analytical methodologies, sample preparation protocols, contamination-control procedures, and tissue matrices analyzed [15]. Furthermore, discrepancies between studies investigating similar tissues may reflect not only methodological variability but also differences in environmental exposure among study populations [21]. MPs themselves are highly heterogeneous with respect to size, shape, polymer type, surface erosion, and physicochemical behavior. As a result, each analytical technique provides distinct advantages and limitations, while combining complementary analytical approaches may provide more comprehensive characterization. The present narrative review aims to summarize the currently available methods for detecting MPs in human tissues, discuss their methodological strengths and limitations, and evaluate their applicability to vascular and intravascular tissue analysis.

Although the primary focus of this review is the detection of micro- and nanoplastics in vascular and intravascular human specimens, the currently available literature specifically addressing vascular tissues remains limited. Consequently, selected studies involving other human biological tissues – including liver, spleen, placenta, cardiac tissue, and transplant specimens – were also considered where they provided important methodological insights applicable to vascular and intravascular sample analysis. Given the evolving nature of the field and the absence of standardized detection protocols, the present review adopts a meth-

od-oriented approach aimed at evaluating the applicability, strengths, and limitations of currently available analytical techniques across complex human tissue matrices, with particular emphasis on their relevance to vascular systems.

Beyond methodological concerns, the growing detection of MPs and NPs across multiple human tissue matrices has also raised increasing interest regarding their possible biological and pathological implications. Emerging literature has suggested potential associations between chronic MP exposure and inflammatory, vascular, metabolic, and degenerative processes, although the underlying mechanisms and clinical relevance remain incompletely understood [27]. These broader concerns further emphasize the importance of developing reliable and standardized analytical methods capable of accurately characterizing microplastic accumulation within human tissues and vascular systems.

To review the literature concerning MPs and the currently available methods of detection and characterization, we searched publications indexed in PubMed and Google Scholar without restriction regarding publication date. Search terms included “microplastics”, “nanoplastics”, “characterization”, “atheroma”, “venous tissues”, “vascular tissues”, “arterial tissues”, “human”, “Raman spectroscopy”, “FTIR”, “Py-GC/MS”, “AFM”, “electron microscopy”, and “fluorescence microscopy”,

used in various combinations. Studies not directly related to the detection or characterization of MPs in human tissues were excluded.

Animal and *in vitro* studies were generally excluded to maintain the clinical and translational focus of the review. However, selected experimental studies investigating the interaction of MPs and NPs with vascular tissues, endothelial barriers, or biological transport mechanisms were included where they provided important mechanistic insight relevant to human vascular and intravascular specimens. Such studies were considered particularly valuable in areas where direct human evidence remains limited. Reference lists of all eligible articles were also screened manually to identify additional relevant publications. Although the present work is structured as a narrative review, a flowchart summarizing the study selection process is provided in Figure 1 to enhance methodological transparency and illustrate the overall approach used for literature identification and selection.

Human tissue matrices investigated for microplastic detection

Blood and thrombotic specimens

Blood and thrombotic specimens represent some of the most direct evidence of the pres-

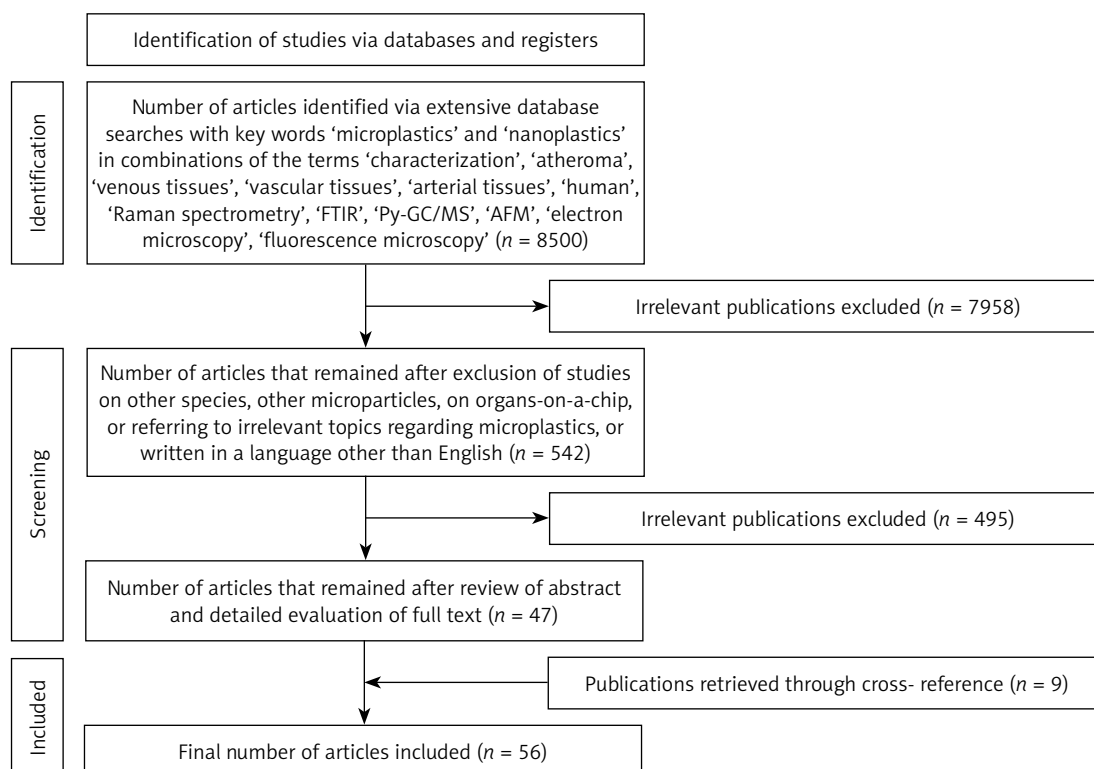


Figure 1. Flowchart of article selection process. A total of 8500 records could be retrieved through database searching, of which 47 were deemed relevant after full-text assessment of 495 publications. 56 studies were included in the final narrative synthesis. It should be noted that we were able to find only 6 studies referring specifically to vascular specimens from humans

ence of MPs within the systemic circulation and vascular system in humans. Compared with solid tissues, these matrices provide important insight into both ongoing exposure and the potential transport pathways of MPs and NPs throughout the body. Several analytical techniques have already been applied to circulating blood and thrombotic material, including pyrolysis–gas chromatography/mass spectrometry (Py-GC/MS), Raman spectroscopy (RS), laser direct infrared spectroscopy (LDIR), and scanning electron microscopy (SEM) [21, 22, 25].

One of the first studies demonstrating the presence of MPs in human blood was conducted by Leslie *et al.* [25], who utilized Py-GC/MS with extensive contamination-control protocols to identify plastic polymers in venous blood samples from healthy volunteers. The study highlighted the suitability of mass-based analytical methods for detecting MPs independently of particle morphology, while also emphasizing the challenges posed by low polymer mass concentrations and environmental contamination. Subsequent investigations expanded these findings by applying multimodal analytical approaches to thrombotic specimens retrieved from patients with acute vascular events [21, 22].

Particularly important are studies involving arterial and venous thrombi, as these specimens may provide mechanistic insight into the interaction between MPs and thrombogenesis. Wang *et al.* [21] combined Py-GC/MS, LDIR, and SEM to analyze thrombi from multiple anatomical sites, demonstrating widespread presence of MPs and an association between higher MP burden and markers of thrombotic severity. Similarly, Wu *et al.* [22] employed Raman spectroscopy to identify microparticles and MPs in thrombotic material obtained during emergency cardiovascular procedures. Although the number of detected MPs remained limited in some specimens, these studies demonstrated the feasibility of applying spectroscopic and multimodal techniques to highly heterogeneous intravascular matrices.

Blood-based and thrombus-based studies also illustrate several important methodological considerations relevant to vascular research. Circulating blood is particularly vulnerable to contamination originating from collection systems, intravenous equipment, laboratory handling, and airborne particles [25–28]. In addition, thrombotic specimens are structurally heterogeneous, containing fibrin, inflammatory cells, calcific debris, cholesterol crystals, and pigment particles, all of which may interfere with spectroscopic characterization and contribute to false-positive or ambiguous signals [21, 22].

An additional consideration relevant to vascular and intravascular research is the potential

contribution of medical exposure pathways to circulating microplastic burden. Recent investigations have suggested that intravenous infusions and medical fluid administration systems may represent a direct source of microplastic exposure, potentially bypassing gastrointestinal and respiratory barriers altogether [28]. Casella *et al.* highlighted the presence of microplastic particles within infusion-related systems and emphasized the possible implications of repeated intravenous exposure in hospitalized patients [29]. Although the clinical significance of these findings remains uncertain, they further underline the importance of rigorous contamination-control protocols and careful interpretation of intravascular microplastic detection studies, particularly in perioperative and critical care settings. Consequently, studies of vascular and intravascular specimens increasingly rely on complementary multimodal approaches rather than isolated analytical techniques alone.

Vascular and atherosclerotic tissues

Among all human tissue matrices investigated for MP presence, vascular and atherosclerotic tissues are of particular interest because they may provide information on the potential interaction between MPs and cardiovascular pathology. Unlike circulating blood, which reflects dynamic transport, vascular wall specimens offer information regarding local MP presence, potential retention, endothelial interaction, and possible involvement in inflammatory and thrombotic processes [20, 21, 30]. Existing studies in this field remain limited in number, but they have already demonstrated the feasibility of detecting MPs and nanoplastics within atherosclerotic plaques, arterial wall tissue, and diseased vascular specimens using a range of complementary analytical techniques.

One of the most notable studies to date was reported by Marfella *et al.* [20], who investigated carotid atherosclerotic plaques obtained during carotid endarterectomy. Utilizing a multimodal approach involving Py-GC/MS, stable isotope analysis, electron microscopy, enzyme-linked immunosorbent assay (ELISA), and immunohistochemical techniques, the authors identified MPs and nanoplastics in a substantial proportion of plaque specimens and reported an association between their presence and an increased risk of adverse cardiovascular outcomes. Importantly, electron microscopy enabled visualization of nanoplastics within macrophages and inflammatory regions of the plaque, suggesting that MPs may interact with cellular and inflammatory components of atherosclerotic plaques rather than merely passively accumulate within vascular tissue [20].

Additional evidence of higher MP concentrations in diseased vascular tissues was provided

by Liu *et al.* [29], who compared atherosclerotic coronary and carotid arteries with non-atherosclerotic aortic specimens using Py-GC/MS. The investigators reported significantly higher MP concentrations in atherosclerotic tissues, supporting the hypothesis that factors associated with vascular disease, such as chronic vascular injury, endothelial dysfunction, altered hemodynamics, and inflammatory remodeling, may facilitate MP retention within diseased arterial walls. However, because Py-GC/MS is a destructive mass-based method, the study was unable to provide information regarding particle morphology, spatial localization, or microstructural interaction with surrounding tissue [30].

These vascular studies also illustrate the importance of combining analytical techniques when investigating complex arterial specimens. Atherosclerotic plaques are highly heterogeneous biological matrices composed of lipid cores, calcification, collagen, inflammatory infiltrates, cholesterol crystals, necrotic debris, and intraplaque hemorrhage, all of which may interfere with spectroscopic or imaging analyses. Consequently, multimodal approaches integrating spectroscopic, chromatographic, and microscopic methods may be particularly valuable for vascular tissue analysis [20, 21, 31, 32]. The limited number of currently available vascular studies further highlights the need for standardized protocols specifically adapted to arterial and intravascular specimens, especially regarding contamination control, tissue digestion strategies, and differentiation between endogenous biological structures and exogenous polymer particles.

Cardiac and perivascular tissues

Cardiac and perivascular tissues constitute another important category of human specimens investigated for the presence of MPs, particularly because they may provide information on both systemic dissemination and local tissue accumulation within the cardiovascular system. Compared with blood or thrombotic material, these tissues provide a more stable substrate for evaluating long-term exposure and possible tissue-specific deposition patterns. Existing studies have primarily focused on specimens collected during cardiac surgery, including myocardium, pericardium, epicardial adipose tissue, left atrial appendage tissue, and perioperative venous blood [33].

Yang *et al.* [32] performed one of the first comprehensive investigations specifically addressing MPs in human cardiac surgical specimens. Using a combination of LDIR and SEM, the authors identified multiple MP polymer types across several cardiac and pericardial tissues obtained intraoperatively from patients undergoing cardiac

surgery. MPs were detected not only in venous blood but also within myocardial and adipose tissue samples, providing evidence of MP presence in multiple cardiovascular tissue compartments. The identification of polymethyl methacrylate and other polymer species demonstrated the presence of multiple polymer types but does not, by itself, establish repeated or chronic environmental exposure [33, 34].

Studies involving cardiac and perivascular tissues also demonstrate the importance of specimen-specific methodological adaptation. Cardiac tissues are rich in extracellular matrix components, adipose tissue, contractile proteins, and blood-derived elements, all of which may complicate tissue digestion, spectroscopic analysis, and polymer identification. In addition, perioperative sampling introduces unique contamination concerns due to the extensive use of polymer-containing surgical equipment, drapes, tubing systems, and extracorporeal circulation devices within the operating theatre environment [28]. Consequently, rigorous contamination-control protocols and multimodal validation strategies are particularly important in studies involving surgically retrieved cardiovascular specimens.

Although the number of available studies remains small, investigations of cardiac and perivascular tissues provide an important link between the detection of MPs in circulating blood and their presence within cardiovascular tissues. These findings further support the need for standardized analytical frameworks specifically tailored to cardiovascular surgical specimens, particularly in relation to tissue digestion protocols, contamination minimization, and multimodal polymer confirmation.

Non-vascular human tissues relevant to methodological validation

Although the primary emphasis of this review is placed on vascular and intravascular specimens, studies involving other human tissues have contributed substantially to the methodological development and validation of MP detection techniques later applied to cardiovascular matrices. Given the limited number of available vascular-specific investigations, evidence derived from non-vascular human tissues remains important for understanding the strengths, limitations, sensitivity, and reproducibility of currently available analytical methods [12, 15, 16]. These studies provide insight into the detection of MPs and nanoplastics within complex tissue environments and their potential ability to cross biological barriers.

Several investigations have demonstrated the presence of MPs in tissues characterized by high vascularization and complex barrier properties,

including the placenta, liver, spleen, kidney, and bone marrow [12, 15–17, 35]. Placental studies are particularly relevant from a methodological perspective because they involve complex microvascular and endothelial interfaces that share some features with vascular tissues. Ragusa *et al.* [16, 35, 36] utilized Raman microspectroscopy, fluorescence-based imaging, and electron microscopy techniques to identify MPs within placental tissue and intracellular compartments, including endothelial-associated structures. These studies highlighted both the strengths and limitations of spectroscopic and microscopic approaches in biologically heterogeneous specimens, particularly regarding contamination control and differentiation between endogenous particles and exogenous polymers.

Liver and spleen tissues have also played an important role in methodological validation because they are highly perfused organs involved in filtration, immune regulation, and systemic particle clearance. Horvatits *et al.* [12] combined fluorescence microscopy with micro-Raman spectroscopy to identify MPs in cirrhotic liver tissue, while other investigations have extended similar approaches to tumor tissues, bone marrow, and transplant specimens [17–19, 35]. These studies demonstrated the feasibility of detecting MPs within dense and structurally complex biological matrices while also underscoring persistent analytical challenges, including tissue digestion requirements, polymer degradation, fluorescence interference, and the risk of environmental contamination.

Importantly, non-vascular tissue studies have frequently provided methodological experience relevant to newer multimodal analytical approaches later applied to vascular and intravascular specimens. Techniques such as combined SEM-Raman workflows, enhanced spectroscopic preprocessing protocols, fluorescence-based imaging strategies, and multimodal chromatographic analyses were often initially optimized in non-vascular tissues before being adapted to thrombi, blood, and vascular wall specimens [31, 32, 37, 38]. Consequently, although these tissues fall outside the strict anatomical definition of the vascular system, their inclusion provides important methodological context for understanding the current capabilities and limitations of MP detection in human cardiovascular tissues.

Taken together, the currently available evidence suggests that microplastic detection in human tissues remains highly dependent on the biological matrix under investigation, with each tissue type presenting distinct analytical challenges related to contamination control, tissue digestion, particle isolation, and polymer identification. These differ-

ences further support the need for matrix-specific methodological standardization, particularly for vascular and intravascular specimens where available evidence remains limited.

Description of available methods

The analytical performance of MP detection methods is strongly influenced by the biological matrix under investigation. Blood, thrombi, vascular wall tissue, adipose tissue, and highly cellular organs each present distinct challenges related to tissue digestion, contamination control, polymer isolation, particle visualization, and spectroscopic interpretation. Consequently, no single analytical technique is currently considered universally applicable across all human tissue matrices. The following sections therefore summarize the principal analytical methods used for microplastic detection, with emphasis on their applicability, advantages, and limitations in relation to vascular and intravascular human specimens.

A summary of the methods that have been used to analyze MPs in humans is presented. Comments on the strengths and weaknesses of each method, as well as an assessment of its utility for the analysis of vascular and intravascular tissues, are provided and listed in Table I.

Stereomicroscopy and fluorescence microscopy

Optical methods based on the interaction of biological specimens with light, including stereomicroscopy and fluorescence microscopy, remain among the most accessible techniques for the preliminary detection and visualization of MPs. These methods are particularly useful for assessing particle morphology, color, and spatial distribution within tissue specimens. However, their analytical capabilities remain limited compared with spectroscopic and chromatographic approaches, particularly with regard to polymer identification and the detection of smaller particles. Because of their relatively high detection limits, these techniques may underestimate the true number and mass of MPs present within biological samples, especially in the submicron range.

Stereomicroscopy allows relatively reliable visual detection of larger MPs, often down to approximately 100 μm , particularly when particles display characteristic shapes, colors, or surface features. In many studies, stereomicroscopy has been combined with Nile Red staining, a lipophilic fluorescent dye with affinity for hydrophobic polymer surfaces [39]. This approach may facilitate rapid preliminary visualization of MPs within undigested tissues before more advanced spectroscopic analysis [40]. Nevertheless, Nile Red is not entirely specific for synthetic polymers and may also stain naturally occurring organic particles and

Table I. Summary of different MP detection and identification methods, their detection limits, and their advantages and disadvantages

Identification method	Particle detection limit	Advantages	Disadvantages
Stereomicroscopy	5–20 μm	- Fast, nondestructive analysis - Data on shape, size, and color - Some data on tissue-MP interaction [39]	- No data on polymer type - Limited for MPs, restrictive minimum detection limit - False detection due to non-plastic particles [38, 41] - Does not detect transparent MPs
Fluorescence microscopy	200–250 nm	- Inherent fluorescence of MPs (especially transparent MPs) [42, 43] - Counterstaining possible [44]	- MP and UV light interference [7] - Photobleaching - Interference with fluorescence from additives [45]
Transmission electron microscopy	0.1–0.5 nm	- High-resolution analysis of MPs and NPs - Detailed analysis of tissue structures	- Heavy-metal staining required - Time-consuming analysis
Scanning electron microscopy	0.5–1 nm	- High-resolution analysis - Provides data about MP aging - Elemental analysis is possible with EDS	- Intensive sample preparation required - Co-analysis with other methods is tedious [31, 32]
Atomic Force microscopy	< 1 nm	- High-resolution analysis - Detailed data about surface structure - Multiple modes of analysis - Information about other physical properties - Analysis under environmental conditions possible - Can be paired with Raman spectroscopy for precise results	- Damage to sample is possible [51] - Cannot exclude contamination [51] - Artifacts possible [51]
Micro-Raman spectroscopy	50–200 nm	- Spatial and material analysis is possible - Non-destructive [55] - Can be utilized for additive analysis - Different modes are available (resonance RS, anti-Stokes RS, etc.)	- Low intensity of Raman signals - Fluorescent interference [38] - Time-consuming - UV degradation of sample MPs [55] - Some mischaracterization of paint particles [22, 58]
Pyrolysis–gas chromatography/mass spectrometry	1–20 μm (mass-based technique, will detect NPs of any size if in sufficient mass in the specimen)	- Measurement regardless of particle size - Qualitative analysis of polymer type possible - Quantitative measurement of polymer mass is possible	- Destructive analysis - Complex data generated - The number or physical characteristics of MPs cannot be measured - MP not measured if below critical mass
Fourier transformed infrared spectroscopy	10–200 μm	- Data about MP composition - No mischaracterization of paint particles [22, 58] - Non-destructive analysis	- Time-consuming - Limited spatial differentiation - Protein interference in biological samples - Aging interference with spectra [38]
Laser direct infrared spectroscopy	10–100 μm	Data about composition and shape characteristics of MPs (not the actual shape) [64]	- Limited spatial differentiation - Narrow spectral range
Fluorescence lifetime imaging microscopy [65]	Typically 200–400 nm	- Material-specific information - Identification of MPs possible directly on tissue/environmental samples - Rapid analysis is possible	- Lack of an MP reference library - The influence of biofilms is unknown
Hyperspectral imaging with enhanced dark-field microscopy [66]	5–10 nm	- Single-cell analysis is possible - Continuous analysis of NP accumulation in live cells possible - Non-destructive	

lipid-rich biological material, potentially leading to false-positive findings [38, 41].

Fluorescence microscopy has also been employed to investigate the ability of MPs and NPs to penetrate biological barriers and localize within tissues [12]. Some MPs exhibit intrinsic fluorescence due to pigments, additives, or degradation products incorporated during manufacturing [42], a phenomenon observed particularly in transparent or lightly colored plastic particles [43]. Combined staining approaches may additionally permit simultaneous visualization of intracellular structures and MPs, thereby providing useful spatial and architectural information regarding particle localization within tissues [44].

Despite these advantages, fluorescence-based methods possess several important limitations. Prolonged ultraviolet exposure may contribute to polymer degradation and photochemical alteration of MPs during analysis. Photobleaching, defined as the gradual reduction in fluorescence signal intensity over time, may further complicate image interpretation and quantitative assessment. In addition, fluorescence emitted by additives, dyes, or endogenous biological material may overlap with polymer-associated signals, thereby reducing analytical specificity [45]. Residual fluorophore within incompletely processed samples may also contribute to background interference and signal distortion [46]. Consequently, fluorescence microscopy is generally best utilized as a complementary visualization technique rather than a definitive standalone method for MP characterization.

Transmission electron microscopy

Transmission electron microscopy (TEM) allows high-resolution imaging of samples containing MPs through the transmission of an electron beam across ultrathin biological specimens. Owing to its nanometer-scale resolution, TEM is particularly useful for visualizing NPs and assessing their intracellular localization within tissues. However, an important limitation of this method is the need for extensive sample preparation and the use of heavy-metal stains to improve contrast [47]. These preparation steps may alter or obscure polymer-associated structures and complicate interpretation, making TEM less suitable as a standalone method for definitive MP characterization.

Nevertheless, TEM has already provided important insights into the interaction of MPs and NPs with vascular tissues. Marfella *et al.* visualized nanoplastics both inside and surrounding foamy macrophages within carotid atherosclerotic plaques obtained from asymptomatic patients undergoing carotid endarterectomy [20]. Their findings suggested that nanoplastic accumulation

within atheromatous tissue may exceed that of larger MPs. TEM has also been used to identify MPs within endothelial cells and pericytes of placental capillaries with great structural detail [48], further supporting the ability of MPs and NPs to penetrate biological barriers and localize within microvascular tissues. In some studies, TEM additionally enabled visualization of luminal narrowing and structural alterations associated with particle deposition

Scanning electron microscopy

Scanning electron microscopy (SEM) utilizes a focused electron beam to scan the surface of a biological specimen. SEM provides high-resolution morphological information regarding MPs, allowing differentiation between fibrous, spherical, hexagonal, and irregularly shaped particles. It may also reveal signs of particle aging and environmental degradation through the visualization of surface imperfections such as cracks, pits, and erosive changes. In addition, SEM is capable of detecting NPs at substantially higher resolution than conventional optical microscopy. However, SEM alone does not provide reliable information regarding polymer composition or particle color, and therefore is generally most valuable when combined with complementary analytical techniques [49].

When coupled with energy-dispersive X-ray spectroscopy (EDS), SEM additionally permits elemental analysis through the detection of X-rays generated during electron beam–sample interactions. This combined approach may improve differentiation between biological structures and exogenous particles and provide additional information regarding particle composition.

A major limitation of SEM is the extensive sample preparation required for biological specimens and polymer-containing tissues, including purification, fixation, dehydration, and conductive coating. Such preparation may alter the specimen and may limit the possibility of applying additional analytical techniques to the same sample. This issue is particularly relevant in vascular tissues and thrombi, which are structurally heterogeneous and may demonstrate considerable regional variability in MP distribution. As a result, different portions of the same specimen may contain markedly different local MP concentrations.

To address some of these limitations, several groups have developed integrated systems enabling near-simultaneous SEM and Raman spectroscopy (RS) analysis within the same sample chamber [31, 32]. These multimodal approaches allow more comprehensive morphological and chemical characterization of MPs while minimizing sample transfer and contamination risk. Neverthe-

less, combined techniques also introduce additional technical challenges, including the need for rapid scanning to avoid particle degradation, low signal-to-noise ratios, and difficulties distinguishing polymer-associated Raman signals from signals arising from C–H-containing biological molecules.

For these reasons, SEM is most commonly used as a complementary rather than stand-alone method in studies investigating MPs in vascular and human tissue specimens [21, 30, 33].

Atomic force microscopy

Atomic force microscopy (AFM) utilizes a specially engineered probe with an ultrafine tip that interacts with the specimen surface through van der Waals, capillary, electrostatic, and frictional forces. These interactions are translated into measurable signals and mapped within a Cartesian coordinate system. Depending on specimen characteristics, AFM may operate in contact, non-contact, or tapping mode, with tapping and non-contact modes potentially reducing the risk of direct damage to softer biological materials [50].

Unlike SEM, AFM does not require conductive coating or surface metallization for the analysis of non-conductive polymers such as MPs. This allows nanoscale surface characterization while preserving many native specimen properties. In addition, AFM can operate under ambient environmental conditions and in liquid media, eliminating the need for vacuum conditions. Beyond topographic imaging, AFM may also provide information regarding stiffness, hydrophobicity, adhesion, and conductivity of analyzed particles and tissues.

Despite these advantages, AFM remains susceptible to contamination and imaging artifacts, particularly those related to probe-tip geometry and sharpness [51]. Similar to other analytical approaches, AFM alone is generally insufficient for definitive MP characterization and is therefore most effective when combined with complementary techniques. One of the most informative combinations is tip-enhanced Raman spectroscopy (TERS), which can substantially improve the spatial resolution of conventional Raman spectroscopy while providing complementary chemical information alongside AFM-derived structural data. Further integration with resonance Raman techniques may provide even more precise nanoscale characterization of MPs and NPs.

Raman spectrometry and micro-Raman spectroscopy (also known as Raman microscopy)

Raman spectrometry (RS) is based on the detection and measurement of Raman scattering, in contrast to Rayleigh scattering, following expo-

sure of a sample to a monochromatic light source. Raman scattering refers to the inelastic scattering of photons, resulting in Stokes and anti-Stokes spectral shifts with frequencies different from that of the incident light. Because each molecule possesses a characteristic Raman spectrum, the resulting signal can serve as a chemical fingerprint. Comparison of the obtained spectra with established spectral databases enables both qualitative and quantitative analysis of compounds within a sample. RS is particularly attractive for MP analysis because the results are largely independent of sample volume [52], and the method is capable of detecting analytes present at relatively low concentrations [53].

Micro-Raman spectroscopy (μ RS) combines Raman spectral analysis with the spatial resolution of optical microscopy, thereby enabling simultaneous chemical and morphological characterization of particles. This makes μ RS particularly useful for the identification of carbon-based nanoparticles and small MPs [54, 55]. An additional advantage is that the method is non-destructive, allowing specimens to undergo further analysis using complementary techniques.

Studies involving environmental and marine specimens have demonstrated that the combined use of RS and Fourier-transform infrared spectroscopy (FTIR) provides greater analytical accuracy than infrared spectroscopy alone. RS is subject to the Abbe diffraction limit [56], but can enable the detection of smaller particles than many alternative spectroscopic techniques. Differences in analytical sensitivity have also been observed between polymer types. RS appears more sensitive for polyvinyl chloride (PVC), whereas FTIR may perform better for certain polyester materials. In addition, RS provides valuable information regarding pigments and fillers within MPs. Misclassification may nevertheless occur, particularly with paint particles, which can sometimes be more reliably differentiated using FTIR. Consequently, the two methods are generally considered complementary. Based on currently available evidence, FTIR appears most suitable and cost-effective for MPs ranging from approximately 50 to 500 μ m, whereas RS is particularly advantageous for particles between 1 and 50 μ m, with detection limits approaching 100 nm in selected experimental settings [57].

Horvatits *et al.* developed a combined fluorescence microscopy and μ RS approach for the detection of MPs ranging from 4 to 30 μ m in human liver and spleen specimens [12]. Although this method successfully identified MPs within digested tissue samples, it did not provide information regarding the precise spatial localization of particles within intact tissue structures.

Wu *et al.* were among the first to report the presence of MPs in thrombi retrieved from patients undergoing emergency surgery for arterial dissection or acute lower-extremity arterial embolism [22]. In this study, RS analysis was complicated by surface weathering of MPs, which reduced spectral peak intensity and interfered with particle identification. Additional difficulties arose from pigments and other microparticles exhibiting spectra similar to those of MPs. This distinction between MPs and non-polymeric microparticles remains an important challenge in Raman-based analysis, particularly because pigments and environmental particles may be present in higher concentrations than MPs within biological specimens [22, 58].

Despite limitations such as weak Raman signal intensity, fluorescence interference, and potential UV-induced degradation of MPs during analysis, RS remains an important technique for MP characterization. Its high spatial resolution offers a significant advantage for the detection of smaller MPs that may be more biologically relevant despite their relatively low total mass [59]. Further developments, including resonance Raman spectroscopy (RRS) and coherent anti-Stokes Raman spectroscopy (CARS), have improved signal amplification and analytical sensitivity, thereby enhancing the overall utility of Raman-based approaches for MP detection.

Pyrolysis–gas chromatography/mass spectrometry

Pyrolysis–gas chromatography/mass spectrometry (Py-GC/MS) is a chemical analytical method that thermally decomposes complex analytes at high temperatures, producing low-molecular-weight fragments that can subsequently be separated by gas chromatography and analyzed by mass spectrometry. This technique has been utilized by several research groups investigating MPs in human tissues. In a landmark study, Leslie *et al.* aimed to detect five polymer types in whole-blood specimens obtained from healthy volunteers [25, 60]. Using filters and blood-draw needles that allowed detection of particles ranging from 0.5 to 500 μm , the authors provided some of the first evidence of circulating MPs in human venous blood.

More recently, Py-GC/MS was employed to compare MP concentrations in atherosclerotic coronary and carotid arteries with those found in non-atherosclerotic aortic tissue [30]. The investigators reported significantly higher MP concentrations in atherosclerotic vessels than in non-atherosclerotic specimens (coronary arteries: 156.50 ± 42.14 vs. 76.26 ± 14.86 $\mu\text{g/g}$ tissue, $p = 0.039$; carotid arteries: 133.37 ± 60.52 vs. 76.26 ± 14.86

$\mu\text{g/g}$ tissue, $p = 0.015$) [30]. These findings support the hypothesis that MP burden may be higher in diseased vascular tissues.

An important advantage of Py-GC/MS is its ability to identify polymer composition and quantify analyte concentration regardless of particle size. However, because the method is destructive, it does not provide information regarding the number, morphology, or physical characteristics of individual particles. As a result, Py-GC/MS is most suitable for confirming the presence of specific polymer types and quantifying cumulative MP mass within a specimen, provided that the polymer concentration exceeds the analytical detection limit.

Several studies have therefore combined Py-GC/MS with complementary methods such as SEM and LDIR to obtain additional morphological and structural information regarding detected MPs. Wang *et al.* recently utilized this multimodal approach to analyze arterial and venous thrombus specimens, evaluating not only MP concentration but also particle number, size, shape, and density [21]. Using LDIR and SEM, the authors identified MPs ranging from 20 to 500 μm and reported an association between increased MP burden and greater disease severity.

Fourier transformed infrared spectroscopy

Fourier-transform infrared spectroscopy (FTIR), particularly micro-FTIR (μFTIR), was among the earliest techniques applied to the detection and characterization of MPs. This method utilizes the infrared absorption spectrum of a sample to provide information regarding its molecular composition, thereby enabling both detection and polymer identification. One of the principal limitations of FTIR is its relatively restricted spatial resolution, which typically limits reliable analysis to particles larger than approximately 20 μm . In addition, proteins and other biological components within tissue specimens may interfere with particle detection and spectral interpretation [21, 61]. Environmental weathering and surface erosion of MPs may further alter infrared spectra and complicate polymer characterization. These limitations suggest that FTIR is often most effective when combined with complementary analytical techniques.

Advances in infrared spectroscopy and microscopy have led to the development of alternative approaches, including quantum cascade laser infrared spectroscopy (QCL-IR), which may provide comparable or improved analytical performance [62]. Unlike conventional FTIR, QCL-IR utilizes monochromatic infrared light rather than a broad spectral range, potentially improving both analysis speed and signal-to-noise ratio. However, its narrower spectral range limits its independent ap-

plicability, and it is therefore generally considered best suited as an adjunct to conventional FTIR-based analysis.

Laser direct infrared spectroscopy

Laser direct infrared spectroscopy (LDIR) is a chemical analytical method utilizing quantum cascade laser technology, with a tunable monochromatic laser beam operating within a selected infrared range. Compared with conventional FTIR, LDIR enables more rapid and spatially precise automated analysis. In addition to polymer identification, LDIR allows quantification of MPs according to particle number within a given sample. It may also provide information regarding certain morphological characteristics, including circularity, solidity, and eccentricity, without requiring manual inspection of each particle, although it does not fully characterize particle shape [63, 64]. Yang *et al.* utilized both LDIR and SEM to analyze cardiac tissue and venous blood specimens from patients undergoing cardiac surgery [32].

Limitations of LDIR include the relatively narrow spectral range analyzed and reduced sensitivity for particles smaller than approximately 20 μm . For this reason, LDIR is often best combined with methods such as Py-GC/MS, which can detect and quantify polymer mass independently of particle size, thereby reducing the risk of underestimating smaller MPs. Conversely, certain polymers present in very low cumulative mass but with particle diameters greater than 20 μm may be detected by FTIR, LDIR, or Raman spectroscopy but remain below the detection limit of Py-GC/MS. These considerations further support the importance of multimodal analytical approaches. A recent study investigating the MP burden in kidney transplant tissue demonstrated the value of combining Py-GC/MS, LDIR, and SEM to improve both chemical and morphological characterization of MPs [35].

Additional methods

As the field of MP research continues to evolve, novel approaches for particle detection and characterization are increasingly being explored. The currently available analytical techniques may eventually be complemented by emerging methods such as fluorescence lifetime imaging microscopy (FLIM) and hyperspectral imaging with enhanced dark-field microscopy (HSI-EDF).

FLIM is based on measuring the time required for an excited fluorophore to return to its ground state, a property that may differ between materials and particles. A recent pilot study by Wohlschläger *et al.* demonstrated preliminary but promising results regarding the rapid detection of MPs in en-

vironmental samples [64]. Hyperspectral imaging combined with enhanced dark-field microscopy increases spectral resolution at the level of individual pixels while remaining non-destructive to the specimen. This technique may therefore provide high-resolution spatial and chemical analysis with relatively rapid acquisition times [65, 66].

Although these methods remain at an early stage of application in human tissue analysis, they illustrate the ongoing expansion of analytical possibilities within MP research and may contribute to future multimodal approaches for vascular and intravascular specimen characterization [66].

Additives analysis

The presence of additives and the possibility of sample contamination complicate all currently available analytical methods and therefore require careful consideration. Analysis of additives such as plasticizers, stabilizers, dyes, and flame retardants remains outside the scope of many MP studies despite their potential biological relevance. The situation is further complicated by the ability of MPs to adsorb environmental pollutants, including heavy metals and organic contaminants, which may be present alongside MPs and contribute to their potential biological effects within the specimen.

Detection and quantification of additives are particularly challenging because these compounds are often present in very low concentrations and may leach from the polymer matrix through diffusion, photodegradation, or environmental aging of MPs [25]. Their physicochemical properties may also differ substantially from that of the parent polymer during analytical processing, especially in the case of NPs. In addition, extraction of additives from biological specimens presents its own methodological difficulties, and even when additives are successfully isolated, it may remain uncertain whether they originated directly from MPs or from other environmental or endogenous sources.

Nevertheless, additives may account for up to 35% of total plastic mass [67], and their biological effects may contribute to MP-related pathophysiological processes. Arsenic represents one notable example because of its established association with cardiovascular complications [68]. For this reason, separate analytical strategies specifically targeting additives and adsorbed pollutants may be essential for obtaining a more comprehensive understanding of the biological effects of MPs within vascular and other human tissues [69].

Contamination concerns

Another major challenge in MP research is the elimination and control of contamination originat-

ing from external sources. This issue is particularly relevant in studies involving vascular and intravascular tissues. Collection protocols for specimens intended for MP detection should ideally be plastic-free or, at minimum, designed to minimize plastic exposure. In practice, this means that all materials, instruments, and solutions used during sampling and processing should undergo appropriate blank or control testing in parallel, without exposure to the biological specimen itself. Such controls help identify contamination originating from airborne deposition, laboratory handling, or procedural materials, even in protocols specifically designed to minimize plastic exposure. None of the currently available analytical methods are entirely immune to contamination, and minimizing false-positive findings remains a major methodological priority [28].

An additional concern relates to the environment in which vascular and intravascular tissues are typically obtained, namely operating theatres and other surgical settings. These environments contain multiple potential sources of airborne and contact contamination, including fibers released from surgical gowns, masks, drapes, tubing systems, and disposable instruments. Although complete elimination of plastic-containing materials from surgical practice is often impractical, reducing unnecessary polymer exposure during specimen collection may help limit contamination risk [70].

Intravenous infusions represent another important potential source of intravascular MP exposure and a potential source of contamination during specimen collection. Many medications are stored in polymer-based containers, administered through plastic infusion systems, and delivered via plastic intravenous cannulas [71, 72]. At present, controlling for this source of MP exposure remains particularly difficult. One possible strategy may involve comparing venous blood samples collected before and after procedures using carefully controlled sampling protocols. Once specimens are transferred to the laboratory, strict contamination-control procedures should continue throughout handling, storage, preparation, and analysis, including the use of non-plastic or low-contamination equipment wherever feasible.

Limitations

This narrative review has several limitations that should be acknowledged. Although a narrative approach allows broader and more flexible synthesis across different analytical methodologies and biological matrices, it lacks the methodological rigor of a systematic review and may therefore be more susceptible to selection bias.

A further limitation of the currently available literature is the relatively small number of studies

specifically investigating vascular and intravascular human tissues, which necessitates partial reliance on methodological evidence derived from broader human tissue matrices.

The studies included in this review, as summarized in Table II, utilized different detection and characterization techniques across a variety of tissue types and biological specimens. As a result, direct qualitative and quantitative comparison between studies remains difficult at the present stage of research. Additional limitations include the relatively small number of high-quality studies currently available, as well as the limited sample sizes analyzed in many investigations.

Another important consideration is that most vascular specimens examined to date have been obtained from patients with pre-existing vascular disease. This may introduce sampling bias and limit the generalizability of current findings to broader populations. Furthermore, differences in contamination-control protocols, tissue preparation methods, and analytical detection limits and reporting criteria across studies continue to complicate interpretation and reproducibility of results.

See supplementary data

A final concern relates to intrasample heterogeneity. Vascular tissues are among the most structurally heterogeneous biological matrices, particularly in the context of atherosclerotic plaques, calcified vascular lesions, venous valves, and thrombotic material. Thorough characterization of MPs within such complex specimens depends not only on overall particle burden but also on local particle concentration and interactions with surrounding tissue structures. This level of spatial and structural information may be lost when specimens undergo extensive hydrolysis or digestion before analysis.

Lastly, rigorous contamination-control strategies remain essential, as accurate detection and quantification of MPs in the micron and submicron range are highly sensitive to even minimal external contamination during sample collection, preparation, and analysis.

Conclusions

Human activity has led to the widespread dissemination of micro- and nanoplastics throughout environmental and biological systems, with growing evidence supporting their presence within multiple human tissue matrices, including vascular and intravascular specimens. As analytical technologies continue to evolve, the ability to detect, characterize, and quantify increasingly smaller polymer particles within complex bio-

Table II. Summary of studies investigating microplastic detection in human vascular, intravascular, and related biological tissues

No.	Date of publication	Author	Study type	Study population and sample type	Population size	Methods of analysis	Primary endpoint	Results	Limitations
1	March 2024	Marfella <i>et al.</i> [20]	Prospective multicenter observational study	Patients undergoing carotid endarterectomy (asymptomatic) Sample type: carotid plaque specimens	N = 304	Py-GC/MS, stable isotope analysis, EM, ELISA, IHC	Composite of acute cardiovascular incidents in patients with evidence of MP compared with patients without MP evidence	150 patients (49.3%) with evidence of MPs; higher risk of primary endpoint was reported in this population	• Laboratory contamination • Lack of socioeconomic data of participants • Only asymptomatic patients included • Did not account for consumption habits
2	May 2024	Wang <i>et al.</i> [21]	Single-center observational study	Patients undergoing thrombectomy due to IS, MI, or DVT Sample type: arterial emboli and venous thrombi retrieved from cerebral arteries, coronary arteries, and deep veins in the lower extremities	N = 30	Py-GC/MS, LDIR, SEM	Not explicitly stated	• MPs detected in 80% of thrombi • 3/10 target polymers identified • Higher MP burden was associated with disease severity and laboratory peripheral blood parameters, such as D-dimer elevation	• Some polymer types remained undetected • LDIR size detection limit • Small sample size, comparison between thrombosis sites • No exposure factors identified • No correlation between MP burden and incidence of thrombotic events • No correlation was sought between blood and thrombus concentration of MPs
3	May 2024	Liu <i>et al.</i> [29]	Single-center observational study	Sample type: atherosclerotic coronary and carotid arteries, non-atherosclerotic aortas	N = 17	Py-GC/MS	Not explicitly stated	• MP detection in all samples • Higher MP concentrations in atherosclerotic vs. non-atherosclerotic tissues	• Some polymer types remained undetected • Small sample size • Identification with a single technique • No information about MP size or shape
4	Aug 2023	Yang <i>et al.</i> [32]	Single-center observational study	Patients undergoing cardiac surgery Sample type: pericardium, epicardial and pericardial adipose tissue, myocardium, left atrial appendage, pre- and post-operative venous blood	N = 15	LDIR, SEM	Not explicitly stated	9 MP types found across five tissue types • Presence of polymethyl methacrylate indicates exposure to a source containing this polymer	• Small sample size • Contamination concerns

Table II. Cont.

No.	Date of publication	Author	Study type	Study population and sample type	Population size	Methods of analysis	Primary endpoint	Results	Limitations
5	July 2023	Wu <i>et al.</i> [22]	Single-center observational study	Patients undergoing emergency cardiovascular operations Sample type: thrombi	N = 26	RS	Not explicitly stated	• Microparticles were detected in 16 thrombi, including MPs in one thrombus	• Site of thrombus not recorded • Hydrolyzed specimen • Identification with a single technique • Focus on other microparticles • Small sample size
6	May 2022	Leslie <i>et al.</i> [25]	Pilot study	Healthy volunteers	N = 22	Py-GC/MS, targeting 5 polymers, with extensive contamination control protocols	Not explicitly stated	• Py-GC/MS demonstrated adequate analytical performance	• No information about MP size or shape • Several target polymer types were not detected

DVT – deep venous thrombosis, ELISA – enzyme-linked immunosorbent assay, EM, electron microscopy, IHC – immunohistochemical assay, IS – ischemic stroke, MI – myocardial infarct, MPs – microplastics, Py-GC/MS – pyrolysis-gas chromatography-mass spectrometry, RS – Raman spectrometry.

logical environments is expected to improve. At present, however, the field remains limited by the absence of standardized analytical protocols, variability in contamination-control strategies, differences in tissue digestion methods, and the heterogeneous nature of human biological specimens. These challenges are particularly relevant in vascular and intravascular tissues, where complex structural composition and potentially low particle burdens may complicate reliable detection and interpretation. The currently available evidence also remains constrained by the relatively small number of vascular-specific studies, emphasizing the importance of methodological insights derived from broader human tissue research. Future progress in the field will depend on the development of standardized, reproducible, and matrix-specific analytical workflows integrating complementary spectroscopic, chromatographic, and microscopic techniques. Expansion of reference polymer databases, improvement of automated particle recognition systems, and harmonization of contamination-control procedures will be essential for achieving greater comparability across studies. Such advances could facilitate more rigorous investigation of the biological interactions, tissue distribution, and potential pathological effects of micro- and nanoplastics within the human cardiovascular system and beyond.

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Conflict of interest

The authors declare no conflict of interest.

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