Physiol. Res. 73 (Suppl. 3): S685-S695, 2024 *https://doi.org/10.33549/physiolres.935500*

REVIEW

Influence of Micro- and Nanoplastics on Mitochondrial Function in the Cardiovascular System: A Review of the Current Literature

Tobias LERCHNER[1,2], Jan JEDLIČKA[1,2], Michaela KRIPNEROVÁ[3], Jiří DEJMEK[2,4], Jitka KUNCOVÁ[1,2]

[1]Institute of Physiology, Faculty of Medicine in Plzen, Charles University, Czech Republic, [2]Biomedical Centre, Faculty of Medicine in Plzen, Charles University, Czech Republic, [3]Institute of Biology, Faculty of Medicine in Plzen, Charles University, Czech Republic, [4]Institute of Biophysics, Faculty of Medicine in Plzen, Charles University, Czech Republic

Received May 9, 2024
Accepted October 1, 2024

Summary

Mitochondria represent pivotal cellular organelles endowed with multifaceted functionalities encompassing cellular respiration, metabolic processes, calcium turnover, and the regulation of apoptosis, primarily through the generation of reactive oxygen species (ROS). Perturbations in mitochondrial dynamics have been intricately linked to the etiology of numerous cardiovascular pathologies, such as heart failure, ischemic heart disease, and various cardiomyopathies. Notably, recent attention has been directed towards the detrimental impact of micro- and nanoplastic pollution on mitochondrial integrity, an area underscored by a paucity of comprehensive investigations. Given the escalating prevalence of plastic particle contamination and the concomitant burden of cardiovascular disease in aging populations, understanding the interplay between mitochondria within the cardiovascular system and micro- and nanoplastic pollution assumes paramount importance. This review endeavors to elucidate the current albeit limited comprehension surrounding this complex interplay.

Key words

Mitochondria • Nanoplastics • Microplastics • Cardiovascular system • Endothelial function • Oxidative phosphorylation

Corresponding author

J. Kuncová, Institute of Physiology, Biomedical Centre, Charles University, Faculty of Medicine in Plzen, alej Svobody 1655/76, 323 00 Plzen, Czech Republic. E-mail: jitka.kuncova@lfp.cuni.cz

Introduction

The sophisticated interplay between mitochondrial function and the cardiovascular system holds profound implications for human health and disease. Mitochondria, the cellular powerhouses responsible for energy production, play a pivotal role in maintaining cardiovascular homeostasis through various metabolic and signalling pathways [1]. Dysregulation of mitochondrial function has been implicated in the pathogenesis of numerous cardiovascular disorders, including heart failure, ischemic heart disease, and cardiomyopathies [2]. Mitochondrial dysfunction is intricately linked to cardiovascular diseases, presenting both challenges and opportunities in understanding and treating these conditions [2].

Microplastics (MPs), small plastic particles measuring less than 5 micrometers, and nanoplastics (NPs), particles on the nanometer scale, have become ubiquitous contaminants in the environment, including water sources and food chains. Understanding the interaction between MPs/NPs and mitochondrial function within the cardiovascular system is crucial for elucidating the underlying mechanisms of nano- and microplastic-induced cardiovascular toxicity. This article aims to review the current, but scarce literature on the relationship between mitochondrial function, cardiovascular function, and MPs/NPS exposure, highlighting the potential implications for human health

[3]. By examining the intersection of mitochondrial biology, cardiovascular physiology, and environmental toxicology, this research seeks to contribute to a comprehensive understanding of the multifaceted effects of MPs/NPs on cardiovascular functions and pave the way for targeted interventions to mitigate their putative detrimental impact.

Mitochondrial metabolism

Mitochondrial metabolism is a fundamental process crucial for energy transformation, cellular homeostasis, and signalling in eukaryotic organisms. Mitochondria play a central role in generating adenosine triphosphate (ATP) through oxidative phosphorylation (OXPHOS) within the electron transport system (ETS) [4]. This process involves the sequential transfer of electrons through protein complexes embedded in the inner mitochondrial membrane, leading to the generation of a proton gradient that drives ATP synthesis by ATP synthase. Additionally, mitochondria are involved in various metabolic pathways, including fatty acid oxidation, amino acid metabolism, and pyruvate decarboxylation providing acetyl-CoA that enters the tricarboxylic acid (TCA) cycle. Mitochondrial metabolic pathways also provide intermediates for biosynthesis of nucleotides (orotate), fatty acids (malonyl-CoA), cholesterol (acetyl-CoA), amino acids (glutamate, aspartate), glucose (phosphoenolpyruvate), heme (succinyl-CoA), and iron-sulphur clusters [5] and serve as signalling hubs that regulate cellular processes such as apoptosis, calcium signalling, and reactive oxygen species (ROS) generation. Dysregulation of mitochondrial metabolism is associated with numerous disorders, including cardiovascular, metabolic, neurodegenerative diseases, cancer, and aging [6]. Understanding the intricate mechanisms underlying mitochondrial metabolism is therefore essential for elucidating the pathophysiology of these diseases and developing targeted therapeutic interventions. Ongoing research continues to uncover novel aspects of mitochondrial biology, shedding light on how these organelles integrate environmental cues and cellular demands to maintain cellular function and adapt to stressors.

Plastic pollution

Pollution with MPs and NPs poses a significant threat to environmental and human health due to their widespread distribution and persistence in ecosystems. These tiny plastic particles originate from the degradation of larger plastic debris, as well as from the direct release of microplastic-containing products like cosmetics, textiles, and industrial abrasives [7]. The chemical composition of MPs/NPs reflects the use of various plastic items mainly in single-use packaging material. The predominant plastic materials found in the atmospheric fallout are polystyrene (PS), polyethylene (PE) and polypropylene (PP). Less than 10 % of plastic particles in the environment is contributed by polyvinyl chloride (PVC) and polyethylene terephthalate (PET) [8]. MPs and NPs can be found practically everywhere. There are reports of plastic particles in the atmosphere, soil and especially rivers and seas [9]. The concentrations of these particles may be difficult to compare, because they are affected by various factors including human activities, meteorological factors (wind, humidity), indoor vs. outdoor, and urban vs. suburban areas. Luo *et al.* reported urban air deposition samples concentration of 110±96 particles/m^2/day vs. 53±38 particles/m^2/day in suburban areas [10]. Others report concentrations ranging from 2.62 to 6.43 μg/m^3 in soil and 3.08 to 5.45 μg/m^3 in water [9]. Environmental conditions affect the properties of MPs and NPs, with a pronounced influence of pH and various electrolytes that cause deflections in particle surface charge causing changes in aggregating behavior [11]. In sum, all these variables have to be taken into consideration since research on particles does not always reflect particles occurring in the real-world setting.

MPs and NPs can enter aquatic environments through runoff, wastewater discharge, and atmospheric deposition, where they accumulate in sediments, surface waters, and marine organisms [12]. Their small size and large surface area-to-volume ratio make them prone to sorbing and concentrating environmental pollutants such as persistent organic pollutants, heavy metals, and microbial pathogens [13]. Moreover, MPs/NPs can be ingested by aquatic organisms, leading to bioaccumulation and biomagnification along the food chain [14]. Human activities, including aquaculture, and consumption of seafood, can consequently expose humans to MPs/NPs pollution, raising concerns about potential health impacts [15,16]. The adverse effects of MPs/NPs on marine organisms include physical harm, inflammation, reproductive impairment, and disruption of physiological processes [17]. Furthermore, emerging evidence suggests that MPs/NPs may translocate across biological barriers and accumulate in tissues, potentially

causing systemic toxicity and promoting the spread of antimicrobial resistance genes [18]. Addressing the pollution of MPs/NPs requires interdisciplinary efforts encompassing research, policy development, waste management strategies, and public awareness campaigns to mitigate their environmental and health implications.

Humans get exposed to MPs/NPs mainly *via* three different routes: ingestion with subsequent processing in the gastrointestinal system (GIT) or inhalation [19,20] or absorption following dermal contact [21]. Thus next to direct effects to both, the GIT or pulmonary system, MPs/NPs are able to exert systemic effects *via* cell membrane penetration and internalisation [22]. Previous studies suggest that NPs induce intestinal barrier dysfunction *via* ROS production [19] and cross the intestinal barrier [20]. Subsequently, there is well-described evidence of accumulation of MPs/NPs in the human blood and systemic distribution reaching various destinations throughout the body with the potential to exert subsequent systemic effects [23,24]. Accumulation in the liver [21], kidneys [22], brain [23], reproductive organs [24] and the cardiovascular system [25] has been described. However, studies highlighting the impact of MPs/NPs on cardiovascular mitochondrial function in humans, including smaller pivotal or larger epidemiological studies, are currently missing.

So far, toxicity of MPs/NPs has been studied mainly in the reproductive and gastrointestinal systems in a number of *in vivo* (rodent and aquatic models) and *in vitro* studies (cell lines). Only few of them focused on detailed analysis of mitochondrial functions [25]. The major finding across all these studies was the increase in ROS generation that is hypothesized to induce further damage to mitochondria and consequently to the function of the whole cell and organ [18]. *In vitro* experiments on the hepatic, intestinal, and lung cells showed that MPs and NPs decreased mitochondrial membrane potential and ATP production, induced apoptosis through mitochondria-dependent pathway and also triggered mitochondrial fission [26-28]. Oxidative stress, compromised lipid and energy metabolism, impaired mitochondrial turnover, and induction of apoptosis were also revealed in *in vivo* studies using rodent models [25].

Toxicity of micro- and nanoplastics on the cardiovascular system and their relation to the mitochondrial function

Cardiovascular system serves as a connection between first contact systems (GIT and lungs) and the rest of the organism. However, literature data on the effects of MPs/NPs on both heart and vessels are scarce.

Cardiotoxicity of micro- and nanoplastics

Wang *et al.* investigated the impact of NPs on cardiac tissue using both *in vitro* and *in vivo* models [26]. They employed H9c2 and AC16 cardiomyocyte cell lines for *in vitro* experiments and a murine model for *in vivo* analysis, administering NPs at three different doses (3 mg/kg, 6 mg/kg, and 10 mg/kg; administered by oral gavage). Notably, *in vivo* examination revealed the internalization of NPs into cardiomyocytes, consistent with previous observations in other cell types [26]. Distribution to other organs, including the kidneys and liver, was also observed. Despite a reduction in functional parameters such as left ventricular ejection fraction (LVEF), the changes remained within physiological ranges. Treatment with NPs induced structural alterations in mouse myocardium, transitioning from organized architecture without inflammation in the control group to disordered and inflamed cell infiltrations in the NPs-treated group. Additionally, myocardial fibrosis was evident, along with elevated levels of molecular fibrosis markers such as α-SMA upon immunohisto-chemical analysis. Markers of myocardial senescence (p16, p21, and p53) exhibited intense upregulation in the NPs-treated group, indicating premature aging. Furthermore, inflammatory mediators in myocardial tissues, including interleukin 6 (IL-6), interleukin-1β (IL-1β), and tumour necrosis factor-α (TNF-α), were significantly elevated. This inflammatory response corresponded with increased levels of ROS, indicative of oxidative stress in the NPs-treated group [26]. *In vitro* experiments further demonstrated the time and dose-dependent internalization of NPs into cardiomyocytes.

Consistent with prior research [29], Wang *et al.* hypothesized that NPs may induce ROS production through the induction of calcium overload. Given the abundance of mitochondria in cardiomyocytes, the impact of ROS on cardiac viability and function is pronounced [29]. Excessive ROS accumulation led to the release of mitochondrial DNA into the cytoplasm, ultimately resulting in cardiomyocyte senescence.

Wei *et al.* investigated the effects of varying concentrations (0.5, 5, and 50 mg/l; drinking water) of polystyrene MPs in an in vivo murine model over a 90-day period [30]. The study revealed notable morphological alterations, including capillary congestion

and myocardial fiber fracture in groups treated with 5 and 50 mg/l of MPs. Concurrently, mitochondrial cristae disappearance in cardiomyocytes and internalization of MPs into these cells were observed. Moreover, significant oxidative damage in cardiac tissue was evident, characterized by elevated levels of malondialdehyde (MDA) and reduced levels of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) in the 5 and 50 mg/l groups compared to controls [30]. The perturbation of these antioxidant enzymes is particularly significant given their roles: CAT utilizes hydrogen peroxide as a substrate, SOD acts as a scavenger and antioxidant, and GSH-Px functions as an intracellular antioxidant. The heightened MDA levels indicate an increase in free radicals, characteristic of oxidative stress. Additionally, clinical biomarkers such as cardiac troponin I (cTnI) and creatine kinase-MB (CK-MB) exhibited significant elevations following MPs exposure. Furthermore, proinflammatory markers like IL-1β and interleukin-18 (IL-18) were markedly increased in the 50 mg/l group. The authors propose that MPs induce pyroptosis in the heart, mediated by and associated with oxidative stress and inflammation [30].

Lu *et al.* conducted a comprehensive investigation utilizing both *in vitro* and *in vivo* models to examine the impact of PET MPs [31]. In their *in vivo* analysis, four distinct groups were established, one control and three subjected to varying concentrations of MPs (0.5, 5, and 50 µg/ml; drinking water). *In vitro* experiments employed H9c2 cells. Following a 90-day treatment period, mice exposed to the highest concentration (50 µg/ml) exhibited notable vascular congestion and myocardial fiber damage, consistent with prior findings reported by Wang *et al.* [26]. Similarly, cardiac fibrosis was observed exclusively in the 50 µg/ml group, while groups exposed to lower concentrations (0.5 or 5 µg/ml) displayed no signs of fibrosis, vascular congestion, or myocardial fiber disruption. Key indicators of oxidative stress, including MDA, SOD, GSH-Px, and CAT, were significantly altered in response to MPs exposure, with MDA levels elevated and SOD, GSH-Px, and CAT levels reduced in the highest concentration group [31]. This is again in concordance with already discussed literature by Wei *et al.* [30]. *In vitro* experiments revealed a dose-dependent increase in intracellular ROS levels with escalating MPs concentrations, suggesting a perturbation in the redox status of both murine subjects and *in vitro* cardiomyocyte cell lines. The authors postulated that MPs-induced myocardial apoptosis stems from the excessive accumulation of ROS, with evidence pointing to the activation of mitochondria-dependent apoptotic pathways following MPs exposure. Enhanced levels of the pro-apoptotic protein Bax and decreased expression of poly (ADP-ribose) polymerase, caspase-3, and Bcl-2 in myocardial tissue underscored increased rates of apoptosis. Additionally, mitochondrial membrane potential in myocardial cells decreased across all treatment groups [31].

Zhang *et al.* conducted an extensive investigation into the in vivo effects of 40 nm polystyrene NPs particles (PS-NPs) in mice [32]. Inhalation exposure to three distinct concentrations (16 µg/day, 40 µg/day, and 100 µg/day) across three exposure durations (1 week, 4 weeks, and 12 weeks) was administered. The study employed RNA sequencing to elucidate potential cardiotoxic mechanisms following acute, subacute, and subchronic exposure [32]. Partly consistent with previous findings by Wang *et al.* [26], short-term exposure initially did not result in alterations in LVEF; however, a significant decrease in LVEF was noted over time. Left ventricular concentric remodelling was evident upon echocardiographic evaluation [32]. Myocardial fragmentation and fibrosis, akin to earlier reports by Wang *et al.* [26] and Lu *et al.* [31], were observed, alongside mitochondrial swelling and deformation. Augmented levels of cardiac biomarkers such as cardiac troponin T (cTnT), atrial natriuretic peptide (ANP), N-terminal pro-brain natriuretic peptide (NT-proBNP), and lactate dehydrogenase (LDH) were documented [32]. Furthermore, a dose-dependent inflammatory response was evident in myocardial tissue following NPS exposure, alongside perturbations in the balance between oxidation and antioxidant properties, evidenced by alterations in CAT, MDA, GSH-Px, and SOD levels, consistent with previous studies [30,31]. To delve deeper into mitochondrial involvement, transcriptome profiling of mouse heart tissue exposed to NPs was performed [32]. The authors concluded that NPs treatment induced disturbances in the TCA cycle, coupled with mitochondrial damage, which collectively contributed to cardiac dysfunction across varying exposure durations. They postulated that mitochondrial impairment may serve even as a primary driver for NPs-induced cardiac pathology [32].

Duan *et al.* conducted a study to assess the impact of polystyrene NPs on zebrafish embryos [33]. Employing 50 nm NPs, the study administered these particles to zebrafish embryos at 24 h post-fertilization. While the primary focus was to investigate the repercussions of

global warming on NPs toxicity, the study unveiled intriguing insights into the molecular mechanisms underlying NPs-induced effects [33]. Remarkably, Duan *et al.* observed a down-regulation of insulin and branched-amino acid pathways, indicative of the contribution of oxidative stress to developmental processes. Elevated exposure temperatures exacerbated NPs accumulation and heightened OXPHOS within mitochondria, culminating in amplified ROS production. Specifically, the study identified an upregulation of key enzymes involved in OXPHOS complexes I, III, IV, and V, including NADH dehydrogenase, cytochrome c reductase, cytochrome c oxidase, and ATP synthase. Contrary to expectations, elevated temperatures exhibited a protective effect against NP-induced cardiovascular toxicity. This protective effect manifested as heightened myocardial contractility in embryos subjected to elevated temperatures following NPs administration. These findings shed light on the complex interplay between NPs exposure, temperature variations, and cardiovascular responses in zebrafish embryos, warranting further investigation into the underlying mechanisms driving these observed effects [33].

A potential role of mitochondrial dysfunction in MPs/NPs-induced cardiac dysfunction is depicted in Figure 1.

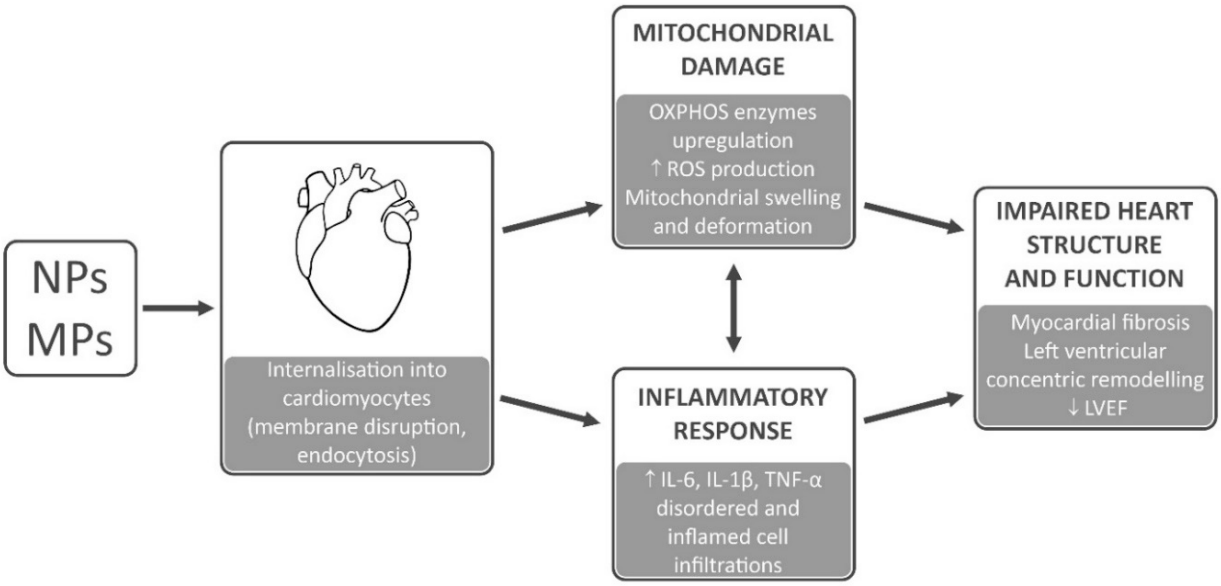

Fig. 1. A possible role of mitochondria in the pathophysiology of MPs/NPs-induced cardiac damage. IL-1β: interleukin-1β; IL-6: interleukin-6; LVEF: left ventricular ejection fraction; MPs: microplastics; NPs: nanoplastics; OXPHOS: oxidative phosphorylation; ROS: reactive oxygen species; TNF-α: tumor necrosis factor α.

Endothelial and vascular toxicity of micro- and nanoplastics

Vlacil *et al.* conducted a thorough investigation into the impact of carboxylated polystyrene MP particles, both *in vivo* and *in vitro* [34]. *In vitro* analyses utilized myocardial endothelial cells and monocytes, crucial players in vascular inflammation. Three concentrations (0.54 ng/ml, 54 ng/ml, and 5.4 µg/ml) were employed for 3- and 6-hour exposures in the cell lines. *In vivo* experiments were conducted in mice receiving 2.5 mg of polystyrene MPs [34]. The authors demonstrated that MPs triggered the expression of inflammatory cytokines IL-1β and TNF-α. Notably, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) were upregulated in endothelial cells, with VCAM-1 released upon MPs exposure. Moreover, increased adhesion of monocytes to endothelial cells served as a marker of pro-inflammatory status following MPs treatment [34]. *In vivo* findings revealed significantly elevated levels of IL-1β and endothelial activation marker VCAM-1. Although ICAM-1 expression in the aortic tissue showed numerical increases post-MPs treatment, statistical significance was not attained [34].

Fu *et al.* conducted an exhaustive exploration into the impact of noncharged polystyrene NPs (PS NPs) and amino-functionalized nanoplastics (NH_2-PS NPs) on the human umbilical vein endothelial cell line (HUVEC) *in vitro* [35]. Their objective was to scrutinize the comparative toxicity of these two NPs variants on HUVEC cells and mitochondria. Concentrations ranging

from 5 to 25 µg/ml of both PS NPs and NH_2-PS NPs were administered, with incubation durations of 12 or 24 h [35]. Following NH_2-PS NPs treatment, a discernible decrease in cell viability and mitochondrial membrane potential, alongside an elevation in ROS levels, were observed. Furthermore, a perturbation in mitochondrial dynamics, replication, and function-related gene expression was noted, predominantly manifesting as a time- and concentration-dependent toxicity for the amino-functionalized particles. Assessment of cell membrane integrity *via* LDH measurement revealed a significant increase in LDH concentration with NH_2-PS NPs compared to noncharged or control groups. Quantitative evaluation of ROS levels through flow cytometry indicated comparable ROS production between NH_2-PS NPs and PS NPs groups, with both exhibiting significantly elevated ROS levels compared to the control group [35]. Fu *et al.* demonstrated that while PS NPs exhibited lower cytotoxicity, they induced higher ROS levels compared to NH_2-PS NPs, suggesting disparate cytotoxic mechanisms. Notably, both NH_2-PS NPs and PS NPs could diminish mitochondrial membrane potential, a phenomenon previously observed by Lu *et al.* [30]. Furthermore, the authors revealed a significant reduction (>50 %) in ATP production capacity of mitochondria treated with both NP variants, correlating with the concentration of plastic particles utilized. In conclusion, the authors inferred that positively charged PS NPs exerted greater toxicity on HUVEC cells, underscoring the pivotal role of surface charge in NPs interactions [35].

So far documented mitochondrial dysfunction induced by MPs/NPs and its relation to vascular damage is shown in Figure 2.

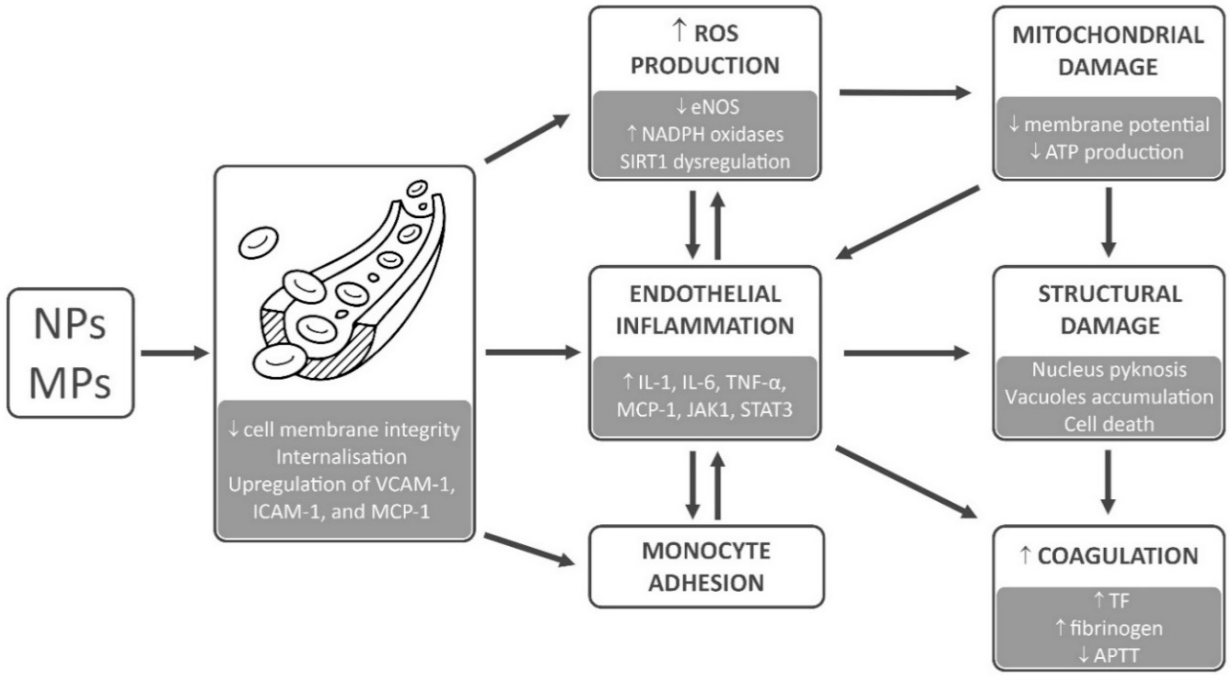

Fig. 2. So far documented features of MPs/NPs-induced vascular damage leading to increase in coagulation. aPTT: activated parcial tromboplastine time; ATP: adenosine triphosphate; eNOS: endothelial nitric oxide synthase; ICAM-1: intercellular adhesion molecule-1; IL-1: interleukin-1; IL-6: interleukin 6; JAK1: Janus kinase 1; MCP-1: monocyte chemoattractant protein-1; MDA: malondialdehyde; MPs: microplastics; NADPH: nicotinamide adenine dinucleotide phosphate; NPs: nanoplastics; STAT3: signal transducer and activator of transcription 3; TF: tissue factor; TNF-α: tumor necrosis factor-α; VCAM-1: vascular cell adhesion molecule-1.

Conclusions

The provided review synthesizes current scientific understanding regarding the toxicological impact of MPs/NPs on mitochondria within the cardiovascular system. Given the pressing concern surrounding plastic pollution and its repercussions on human cardiovascular health, particularly its intricate interplay with mitochondria, literature on this subject remains limited. Until now, no epidemiological studies exist relating exposure to MPs/NPs to mitochondrial function and cardiovascular health in humans.

It has been established that the toxicity of MPs/NPs predominantly exhibits time- and dose-

dependent characteristics [32]. Exposure to plastic particles leads to notable impairment in cardiac and cardiomyocyte integrity and structure [30,31]. Several research endeavors have documented vessel swelling as a consequence of plastic particle exposure. Nevertheless, there is a dearth of data elucidating the direct effects on cardiac function markers such as LVEF, with conflicting findings existing within the literature [26,32]. While some studies do not report a significant alteration in LVEF following exposure to plastic particles, others delineate a dosage-dependent relationship between plastic particle pollution and diminished LVEF [26,32].

MPs/NPs exhibit robust interactions with mitochondrial function. Studies by various authors demonstrate a reduction in mitochondrial membrane potential. Additionally, significant decreases in the activity of mitochondrial complexes I, III, IV, and V, integral components of the inner mitochondrial membrane involved in energy production *via* OXPHOS, have been reported [33]. Elevated ROS production resulting from high-dose plastic particle treatment is a consistent finding across numerous studies, with ROS levels strongly correlated with increased apoptosis.

Furthermore, it remains uncertain whether all plastic polymers exert identical deleterious effects on mitochondrial function within the cardiovascular system. Research by Fu *et al.* suggests that charged particles possess distinct properties compared to uncharged counterparts, thereby warranting further investigation into potential variations in their implications for human health [35].

Given the escalating trends in plastic pollution and cardiovascular disease incidence, there is an imperative need for more comprehensive and detailed research in this area. After elucidating the detailed effects known regarding MPs/NPs impact on mitochondria in the pre-clinical models, this review could lay the groundwork for observational studies in humans.

Key gaps and future direction

The impact of MPs/NPs on mitochondrial function in the cardiovascular system leads to a substantial threat for human health, especially while considering the steady increase in both, the total amount of consumer plastics used and an ageing society with heterogeneous comorbidities. To date, literature highlighting the direct and indirect, as well as short, intermediate- and long-term consequences of everyday exposure to MPs/NPs for mitochondrial function capacity in the cardiovascular system remains scarce. Studies available focus on different pre-clinical models, exposure routes, polymer types and exposure times, lacking homogenous assessment of this pressing topic. By translating findings from different pre-clinical models to studies conducted on humans, including analysis of peripheral blood samples and assessment of confounders like geographics, age and comorbidities, meaningful insights into real-world phenotypes could be generated in the future. Detailed fundamentals of MPs/NPs behavior need to be assessed, both *in vitro* and *in vivo*, to be able to unravel a holistic understanding of everyday exposure to mitochondrial function in the cardiovascular system.

Controversies

Chemical composition of NPs and cell uptake

In many studies, exact chemical composition and toxicology of MPs/NPs is not known. Also, in many studies convincing proof of NPs uptake into cells is missing. Frequent methods for MPs/NPs visualization are flow cytometry or fluorescent microscopy. Using this method may not reliably distinguish whether the MPs/NPs are internalized inside the cells or just adhered on the cell membrane. In the study conducted by Choi *et al.* [36], PS microfragments did not exhibit cellular uptake nor physical damage to the cells. Chemical mechanism of toxicity is being suggested. PS is produced by free radical polymerization of styrene by chemical substances (initiators of polymerization) such as benzoyl peroxide, lauryl peroxide, azobisisobutyronitrile (AIBN) and many more. PS may by further modified by substances such as phenols, mercuric chloride, chlorine, polysubstituted benzene derivatives, hypochlorite, or iodine for antimicrobial treatment [35]. These substances may be released from PS microfragments and serve as the immediate toxin. Exact mechanism of PS production and substances used are usually not known, and in some cases even the subject of production secret.

Duration of exposition

Effects and accumulation of MPs/NPs may be correlated with the duration of exposure. Studies cultivating cells or feeding animals with plastic particles vary from exposure time ranging from mere hours to days or even weeks (Table 1). Chronic experiments (months to years) are scarce. Various physical and chemical properties of the particles are often missing (e.g. particle surface charge).

Table 1. Summary of micro- (MPs) and nanoplastics (NPs) toxicity on mitochondria in the cardiovascular system.

Models	**Material**	**Particle size**	**Exposure length**	**Mechanism**	**References**
Murine myocardial endothelial and immune cells (MyEND cells)	Polystyrene	1 µm	3 h 6 h	*In vitro*: adhesion molecule expression in endothelial cells with subsequent adhesion of leukocytes. Pro-inflammatory cytokine expression and release. *In vivo*: aortic expression of cytokines and adhesion molecules.	Vlacil *et al.* [34]
Human umbilical vein endothelial cell line (HUVEC)	Polystyrene	50 nm	12 h 24 h	*In vitro*: NH_2-PS NPs presented higher risks to endothelial cells than non-charged nanoplastics by interfering with mitochondria.	Fu *et al.* [35]
Murine cardiomyocytes	Polystyrene	40 nm	1 w 4 w 12 w	*In vivo*: PS-NPs induced cardiac injury in a dose-dependent and time-dependent manner.	Zhang *et al.* [32]
Murine cardiomyocytes and H9c2 cardiomyocyte cell line	N/A	20-30 nm	8 w	*In vivo*: nanoplastics induce cardiac aging and senescence. *In vitro*: nanoplastics cause mitochondrial destabilization by inducing oxidative stress.	Wang *et al.* [26]
Zebrafish (Danio rerio) model	Polystyrene	50 nm	24 h	*In vivo*: down-regulation of the branched-chain amino acid and insulin signalling pathways owing to induced oxidative stress.	Duan *et al.* [33]
Wistar rats	Polystyrene	0.5 mm	90 d	*In vivo*: MPs damage cardiac structure and function with impaired mitochondria integrity and triggered oxidative stress.	Wei *et al.* [30]
ICR mice and H9c2 cardiomyocytes	PET	5-10 µm	90 d	*In vivo*: capillary congestion, myocardial fibre breakage, fibrosis, and apoptosis *In vitro*: decreased mitochondrial membrane potential and apoptosis.	Lu *et al.* [31]

HUVEC: human umbilical vein endothelial cell line; MyEND cells: murine myocardial endothelial cells; NH_2-PS NPs: amino-functionalized nanoplastics; h = hours, d = days, w = weeks, PET = Polyethylene terephthalate.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

The study was supported by the Cooperatio Program, Charles University, and by the Specific Student Research Project No. 260653/2023 of Charles University.

Abbreviations

ANP, atrial natriuretic peptide; aPTT, activated parcial tromboplastine time; ATP, adenosine triphosphate; CAT, catalase; CK-MB, creatine kinase-MB; cTnI, cardiac troponin I; cTnT, cardiac troponin T; eNOS, endothelial nitric oxide synthase; ETS, electron transport system; GIT, gastrointestinal tract; GSH-Px, glutathione

peroxidase; ICAM-1, intercellular adhesion molecule-1; IL-1β, interleukin-1β; IL-6, interleukin 6; IL-18, interleukin-18; JAK1, Janus kinase 1; LDH, lactate dehydrogenase; LVEF, left ventricular ejection fraction; MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde; MPs, microplastics; NADPH, nicotinamide adenine dinucleotide phosphate; NH_2-PS NPs, amino-functionalized nanoplastics; NPs, nanoplastics; NT-proBNP, N-terminal pro-brain natriuretic peptide; OXPHOS, oxidative phosphorylation; PS NPs, polystyrene nanoplastics; ROS, reactive oxygen species; α-SMA, α-smooth muscle actin; SOD, superoxide dismutase; STAT3, signal transducer and activator of transcription 3; TCA, tricarboxylic acid; TF: tissue factor; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1

References

1. Bhatti JS, Bhatti GK, Reddy PH. Mitochondrial dysfunction and oxidative stress in metabolic disorders - A step towards mitochondria based therapeutic strategies. Biochim Biophys Acta Mol Basis Dis 2017;1863:1066-1077. https://doi.org/10.1016/j.bbadis.2016.11.010
2. Marin-Garcia J and Akhmedov AT. Mitochondrial dynamics and cell death in heart failure. Heart Fail Rev 2016;21:123-136. https://doi.org/10.1007/s10741-016-9530-2
3. Prata JC. Airborne microplastics: Consequences to human health? Environ Pollut 2018;234:115-126. https://doi.org/10.1016/j.envpol.2017.11.043
4. Wallace DC. Mitochondria and cancer. Nat Rev Cancer 2012;12:685-698. https://doi.org/10.1038/nrc3365
5. Nunnari J and Suomalainen A. Mitochondria: in sickness and in health. Cell 2012;148:1145-1159. https://doi.org/10.1016/j.cell.2012.02.035
6. Raimundo N. Mitochondrial pathology: stress signals from the energy factory. Trends Mol Med 2014;20:282-292. https://doi.org/10.1016/j.molmed.2014.01.005
7. Andrady AL. Microplastics in the marine environment. Mar Pollut Bull 2011;62:1596-1605. https://doi.org/10.1016/j.marpolbul.2011.05.030
8. Allen S, Allen, D, Phoenix VR, Le Roux G, Jiménez PD, Simonneau A, Binet S, Galop D. Atmospheric transport and deposition of microplastics in a remote mountain catchment. Nat Geosci 2019;12:339-344. https://doi.org/10.1038/s41561-019-0335-5
9. Besseling E, Redondo-Hasselerharm P, Foekema EM, Koelmans AA. Quantifying ecological risks of aquatic micro- and nanoplastic. Crit Rev Env Sci Tec 2018;49:32-80. https://doi.org/10.1080/10643389.2018.1531688
10. Luo D, Chu X, Wu Y, Wang Z, Liao Z, Ji X, Ju J, ET AL. Micro- and nano-plastics in the atmosphere: A review of occurrence, properties and human health risks. J Hazard Mater 2024;465:133412. https://doi.org/10.1016/j.jhazmat.2023.133412
11. Pradel A, Catrouillet C, Gigault J. The environmental fate of nanoplastics: What we know and what we need to know about aggregation. NanoImpact 2023;29:100453. https://doi.org/10.1016/j.impact.2023.100453
12. Cole M, Lindeque P, Fileman E, Halsband C, Goodhead R, Moger J, Galloway TS. Microplastic ingestion by zooplankton. Environ Sci Technol 2013;47:6646-6655. https://doi.org/10.1021/es400663f
13. Rochman CM, Tahir A, Williams SL, Baxa DV, Lam R, Miller JT, ET AL. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Sci Rep 2015;5:14340. https://doi.org/10.1038/srep14340
14. Van Cauwenberghe L and Janssen CR. Microplastics in bivalves cultured for human consumption. Environ Pollut 2014;193:65-70. https://doi.org/10.1016/j.envpol.2014.06.010
15. Browne MA, Dissanayake A, Galloway TS, Lowe DM, Thompson RC. Ingested microscopic plastic translocates to the circulatory system of the mussel, Mytilus edulis (L). Environ Sci Technol 2008;42:5026-5031. https://doi.org/10.1021/es800249a
16. Bastyans S, Jackson S, Fejer G. Micro and nano-plastics, a threat to human health? Emerg Top Life Sci 2022;6:411-422. https://doi.org/10.1042/ETLS20220024
17. Wright SL, Thompson RC, Galloway TS. The physical impacts of microplastics on marine organisms: a review. Environ Pollut 2013;178:483-492. https://doi.org/10.1016/j.envpol.2013.02.031

18. Mattsson K, Ekvall MT, Hansson LA, Linse S, Malmendal A, Cedervall T. Altered behavior, physiology, and metabolism in fish exposed to polystyrene nanoparticles. Environ Sci Technol 2015;49:553-561. https://doi.org/10.1021/es5053655
19. Zhang Q, Xu EG, Li J, Chen Q, Ma L, Zeng EY, Shi H. A Review of Microplastics in Table Salt, Drinking Water, and Air: Direct Human Exposure. Environ Sci Technol 2020;54:3740-3751. https://doi.org/10.1021/acs.est.9b04535
20. Sivagami M, Selvambigai M, Devan U, Velangani AAJ, Karmegam N, Biruntha M, Arun A, ET AL. Extraction of microplastics from commonly used sea salts in India and their toxicological evaluation. Chemosphere 2021;263:128181. https://doi.org/10.1016/j.chemosphere.2020.128181
21. Zhu C, Liu S, Cao Z, Hu B, Yang C, Luo X, Yuan H, Li L. Human dermal exposure to short- and medium-chain chlorinated paraffins: Effect of populations, activities, gender, and haze pollution. J Hazard Mater 2024;476:135169. https://doi.org/10.1016/j.jhazmat.2024.135169
22. Lee SE, Yi Y, Moon S, Yoon H, Park YS. Impact of Micro- and Nanoplastics on Mitochondria. Metabolites 2022;12:897. https://doi.org/10.3390/metabo12100897
23. Leslie HA, van Velzen MJM, Brandsma SH, Vethaak AD, Garcia-Vallejo JJ, Lamoree MH. Discovery and quantification of plastic particle pollution in human blood. Environ Int 2022;163:107199. https://doi.org/10.1016/j.envint.2022.107199
24. Geppner L, Ramer G, Tomasetig D, Grundhöfer L, Küss J, Kaup M, Henjakovic M. A novel enzymatic method for isolation of plastic particles from human blood. Environ Toxicol Pharmacol 2023;104:104318. https://doi.org/10.1016/j.etap.2023.104318
25. Guo X, Wang L, Wang X, Li D, Wang H, Xu H, Liu Y, ET AL. Discovery and analysis of microplastics in human bone marrow. J Hazard Mater 2024;477:135266. https://doi.org/10.1016/j.jhazmat.2024.135266
26. Wang K, Du Y, Li P, Guan C, Zhou M, Wu L, Liu Z, Huang Z. Nanoplastics causes heart aging/myocardial cell senescence through the Ca(2+)/mtDNA/cGAS-STING signaling cascade. J Nanobiotechnology 2024;22:96. https://doi.org/10.1186/s12951-024-02375-x
27. DeLoid GM, Cao X, Bitounis D, Singh D, Llopis PM, Buckley B, Demokritou P. Toxicity, uptake, and nuclear translocation of ingested micro-nanoplastics in an in vitro model of the small intestinal epithelium. Food Chem Toxicol 2021;158:112609. https://doi.org/10.1016/j.fct.2021.112609
28. Lennicke C and Cocheme HM. Redox metabolism: ROS as specific molecular regulators of cell signaling and function. Mol Cell 2021;81:3691-3707. https://doi.org/10.1016/j.molcel.2021.08.018
29. Chen YR and Zweier JL. Cardiac mitochondria and reactive oxygen species generation. Circ Res 2014;114:524-537. https://doi.org/10.1161/CIRCRESAHA.114.300559
30. Wei J, Wang X, Liu Q, Zhou N, Zhu S, Li Z, Li X, Yao J, Zhang L. The impact of polystyrene microplastics on cardiomyocytes pyroptosis through NLRP3/Caspase-1 signaling pathway and oxidative stress in Wistar rats. Environ Toxicol 2021;36:935-944. https://doi.org/10.1002/tox.23095
31. Lu T, Li D, Yuan X, Wang Z, Shao Z, Feng X, Yang C, ET AL. Potential Effects of Orally Ingesting Polyethylene Terephthalate Microplastics on the Mouse Heart. Cardiovasc Toxicol 2024;24:291-301. https://doi.org/10.1007/s12012-024-09837-6
32. Zhang T, Yang S, Ge Y, Wan X, Zhu Y, Yang F, Li J, ET AL. Multi-dimensional evaluation of cardiotoxicity in mice following respiratory exposure to polystyrene nanoplastics. Part Fibre Toxicol 2023;20:46. https://doi.org/10.1186/s12989-023-00557-3
33. Duan Z, Wang J, Zhang H, Wang Y, Chen Y, Cong J, Gong Z, ET AL. Elevated temperature decreases cardiovascular toxicity of nanoplastics but adds to their lethality: A case study during zebrafish (Danio rerio) development. J Hazard Mater 2023;458:131679. https://doi.org/10.1016/j.jhazmat.2023.131679
34. Vlacil AK, Bänfer S, Jacob R, Trippel N, Kuzu I, Schieffer B, Grote K. Polystyrene microplastic particles induce endothelial activation. PLoS One 2021;16:e0260181. https://doi.org/10.1371/journal.pone.0260181
35. Fu Y, Fan M, Xu L, Wang H, Hu Q, Jin Y. Amino-Functionalized Polystyrene Nano-Plastics Induce Mitochondria Damage in Human Umbilical Vein Endothelial Cells. Toxics 2022;10:215. https://doi.org/10.3390/toxics10050215

36. Choi D, Bang J, Kim T, Oh Y, Hwang Y, Hong J. In vitro chemical and physical toxicities of polystyrene microfragments in human-derived cells. J Hazard Mater 2020;400:123308. https://doi.org/10.1016/j.jhazmat.2020.123308