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**Impact of Microplastics and Nanoplastics on Liver Health: Current Understanding and Future Research Directions**

microplastics and nanoplastics and liver

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Abstract
With continuous population and economic growth in the 21st century, plastic pollution is a major global issue. However, the health concern of microplastics/nanoplastics (MPs/NPs) decomposed from plastic wastes has drawn public attention only in the recent decade. This article summarizes recent works dedicated to understanding the impact of MPs/NPs on the liver- the largest digestive organ, which is one of the primary routes that MPs/NPs enter human bodies. The interrelated mechanisms including oxidative stress, hepatocyte energy re-distribution, cell death and autophagy, as well as immune responses and inflammation, were also featured. In addition, the disturbance of microbiome and gut-liver axis, and the association with clinical diseases such as metabolic dysfunction-associated fatty liver disease, steatohepatitis, liver fibrosis, and cirrhosis were briefly discussed. Finally, we discussed potential directions in regard to this trending topic, highlighted current challenges in research, and proposed possible solutions.

Key Words: Microplastics; Nanoplastics; Liver; Reactive oxidative species; Cell death; Autophagy; Innate immunity; Metabolic-associated fatty liver disease; Gut-liver axis

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Core Tip: The liver is heavily impacted by exposure to microplastics/nanoplastics (MPs/NPs). This editorial not only summarized the key molecular and cellular events in the liver triggered by MPs/NPs but also highlighted prospective research directions including translational and clinical studies for further investigation in this field.

INTRODUCTION
Plastic pollution has become one of the greatest challenges in the 21\textsuperscript{st} century. The growing use of plastic materials nowadays has caused a serious burden not only to the environment but to human health. Microplastics (MPs, typically $\leq$5 mm) and nanoplastics (NPs, typically $\leq$1 $\mu$m) are small plastic particles manufactured by industry or degraded by physical and chemical processes\cite{1, 2}. These particles are now ubiquitously observed in the soil, drinking water, and even the air we breathe\cite{3}. Furthermore, plastic particles can also enter the food chain and be biomagnified, which finally will return to our dining table and accumulate in the human body\cite{4}. Despite a potential threat to human health, this critical issue has only attracted public attention in recent years. Recent studies have indicated that the occurrence and accumulation of MPs in human body including blood, lungs, liver, and even in human placenta, and received considerable attention\cite{5}. However, biomonitoring, translational and clinical studies of human body burdens of MPs/NPs are still in their infancy.

Among these, the liver is a major organ of the reticuloendothelial system, also known as the monocyte-phagocytic system, which contains gatekeeper cells like sinusoidal endothelial cells or Kupffer cells, capable of clearing foreign particles in blood circulation\cite{6}. In addition, enterohepatic circulation includes the transportation of substances absorbed by enterocytes through portal flow and the passage of bile into the intestine \textit{via} the biliary tracts\cite{7}. This re-entry cycle can cause repeated exposure of MPs/NPs to hepatocytes and sequelae in the liver. Although \textit{in vitro} and \textit{in vivo} studies have demonstrated possible mechanisms that microplastics can affect liver health (Figure 1), human studies are currently limited.

**OXIDATIVE STRESS**

MPs/NPs can either generate extracellular reactive oxygen species (ROS) by weathering degradation like light or heat\cite{8}, or intracellular ROS by disrupting the mitochondrial membrane integrity and potential after internalization\cite{3}. The redox imbalance can further cause DNA damage and genotoxicity, protein oxidation and misfolding, and lipid peroxidation with membrane instability. \textit{Metabolic dysfunction-associated fatty
liver disease (MAFLD), or metabolic dysfunction-associated steatotic liver disease (MASLD) is a liver manifestation of metabolic syndrome which affects nearly one-third of the global adult population\textsuperscript{[9]}. The theory of multiple blows is currently a recognized pathogenesis of MAFLD\textsuperscript{[10, 11]}. Although multiple hits like diet, obesity, insulin resistance, genetic factors, and gut dysbiosis have been found to contribute to MAFLD pathogenesis, environmental toxins or pollutants were barely mentioned in previous literature\textsuperscript{[12, 13]}. Recently, multiple models have demonstrated that the liver can be insulted by microplastics through ROS generation, directly or indirectly resulting in MAFLD. In zebrafish models, combined exposure to a high-fat diet and microplastics increased oxidative stress and upregulated lipogenic and inflammatory gene expression, which led to steatotic liver and altered behaviors\textsuperscript{[14]}. Co-exposure of microplastics with antibiotic pollutants in zebrafish exhibited significantly higher levels of lipid accumulation and inflammation in conjunction with oxidative stress production in their livers\textsuperscript{[15]}. In mice, single-cell transcriptome analysis revealed that microplastics trigger Kupffer cell and T cell activation in the high-fat diet context\textsuperscript{[16]}. The study also showed microplastics regulate PPAR signaling, chemical carcinogenesis-ROS pathways, and complement and blood coagulation cascade in the liver. In human pluripotent stem cell-derived liver organoids, MPs increased the gene and protein expression of hepatic HNF4A and CYP2E1, which control lipid metabolism, insulin signaling, and mitochondrial function\textsuperscript{[17]}. The upregulation of the cytochrome p450 enzyme, CYP2E1, is responsible for the phase I metabolism of the liver and is highly linked to the occurrence of oxidative stress. Activated Kupffer cells can form extracellular traps of MPs/NPs, driving hepatocellular epithelial-mesenchymal transition and pro-inflammatory cytokine production through the ROS signaling pathway\textsuperscript{[18]}

**HEPATOCYTE ENERGY DEPRIVATION**

The energy metabolism affected by MPs/NPs is not merely limited to lipids. Exposure to MPs changes the purinergic metabolites in the liver, which suggests MPs can deplete the energy reserve of different organisms\textsuperscript{[19-21]}. Additionally, the mRNA of nd5, an
important protein subunit of the electron transport chain located at the inner membrane of mitochondria, was altered after exposure to NPs\(^{22}\). Since MPs/NPs can cause mitochondrial damage, it is expected that NPs interfere with the ability to produce ATPs and mobilize energy reserve, which is further echoed by liver and serum metabolite analyses related to tricarboxylic acid cycle and glycolysis\(^{23, 24}\). Moreover, liver transcriptomic and metabolomic studies revealed MPs/NPs can perturb monosaccharide and lipid metabolism including pentose phosphate pathways and gluconeogenesis\(^{25, 26}\). Not only do MPs/NPs inhibit building block synthesis and signal transduction, but they also damage intestinal function and suppress the absorption of nutrients\(^{27}\). Overall, these studies indicate that MPs/NPs can lead to energy deprivation in the liver.

**CELL DEATH AND AUTOPHAGY**

A multitude of evidence suggests MPs/NPs drive cell death including apoptosis, pyroptosis, and ferroptosis. MPs activated hepatic intrinsic apoptosis signaling p53/Bcl-2/Bax signaling\(^{28}\) and meanwhile stimulated the compensatory antioxidant Nrf2/Keap1 pathway\(^{29}\). Besides, studies showed MPs/NPs induced apoptosis by activating PERK and MAPK pathways\(^{30, 31}\). In addition, MPs/NPs induced hepatocyte pyroptosis by increasing NLRP3/ASC and caspase-1 dependent pathway\(^{32, 33}\). Furthermore, MPs induced lipid peroxidation in the liver, which regulates ferroptosis-related proteins such as TFRC, FTH1, and GPX4\(^{32}\). MPs/NPs can also lead to hepatocyte autophagy by altering autophagosome LC3 and p62 ratios\(^{33-35}\), and mitophagy by PERK pathway with increased ER stress\(^{31}\). A recent study demonstrated MPs trigger apoptosis and necroptosis in mouse liver through the ROS/PTEN/PI3K/AKT axis with excessive autophagy flux\(^{36}\).

**IMMUNE RESPONSES AND INFLAMMATION**

MPs/NPs promote inflammation and stimulate innate immune responses. After the 30-day exposure to MPs, the mouse liver showed severe vacuolar degeneration, hepatocyte
edema, and inflammatory cell infiltration[20]. MPs/NPs increase cytokine expression and induce enzymatic activity related to inflammation[37, 38]. The NF-κB pathway is activated, which furthers the inflammatory response in the liver[39]. Exposure to MPs can recruit neutrophils, macrophages, and natural killer cells to the liver[39, 40]. Among the infiltrative immune cells, Kupffer cells (liver-resident macrophages) play a central role in lipid metabolism and responses of hepatocytes to fat overload[41]. The activation of Kupffer cells by engulfing MPs/NPs will affect lipid metabolism, oxidize free fatty acids, and then produce excessive ROS and result in liver damage[41-43]. Furthermore, MPs polarized hepatic macrophages to pro-inflammatory M1 type and facilitated extracellular trap formation of neutrophils and macrophages[18, 39, 40, 44]. Notably, one recent study suggested that polyethylene microplastics impede the innate immune response in the liver by disrupting the extracellular matrix[45]. The contradictory result to previous research may need more future studies to confirm and clarify the underlying mechanism.

FIBROSIS AND CIRRHOSIS

Most chronic hepatitis ultimately results in fibrosis and cirrhosis. One study showed that NPs can increase ROS and exacerbate high-fat diet-induced liver fibrosis[46]. Another study demonstrated the ROS generated by MPs can act on the TGF-β/Smad2/3 signaling axis in hepatocytes[18]. Also, ROS can cause DNA break and release from both hepatocyte nuclei and mitochondria, where in the cytoplasm the fragmented DNA sensing cGAS/STING cascade is triggered and the pro-fibrotic NF-κB pathway is activated[47]. In addition, co-exposure to cadmium and MPs promotes the extracellular release of ATP through the hemichannels of hepatocytes. The extracellular ATP activates hepatic stellate cells by interacting with P2X7 receptors and initiates fibrosis[48]. Interestingly, one retrospective study analyzing human liver tissue discovered six different MP polymers in the liver of individuals with cirrhosis, but not in those without underlying liver disease[49].
FUTURE RESEARCH DIRECTIONS

The pathogenesis of MPs/NPs may appear challenging and complicated offering a lot of research opportunities. Microbiome research has become one of the popular topics in the recent decade. Several studies have uncovered that MPs/NPs disturb the homeostasis of gut microbiota, which affects hepatic fat accumulation and steatohepatitis\textsuperscript{[15, 50, 51]}. In zebrafish models, the abundance of Bacteroidetes and Proteobacteria decreased significantly and the abundance of Firmicutes increased significantly by polystyrene MPs\textsuperscript{[15, 52]}. On the contrary, polystyrene MP exposure decreased the relative abundances of Firmicutes and a-Proteobacteria in mouse intestines\textsuperscript{[51]}. Conflicting results in different species require future studies for validation. However, high throughput sequencing of the 16S rRNA gene V3-V4 region revealed a significant change in the richness and diversity of gut microbiota in both polystyrene MP-exposed zebrafish and mice\textsuperscript{[51, 52]}. MPs/NPs-related dysbiosis may be a "second hit" or be sensitized by other factors to cause intestinal barrier dysfunction (leaky gut) and liver inflammation\textsuperscript{[53-56]}. In addition, MPs/NPs can leach out additives, flame retardants, dyes, and other organic compounds, the adsorbability, large surface area, and biodistribution characteristics of MPs/NPs also can accentuate the bioaccumulation and toxicity of heavy metals and organic compounds (Trojan-horse effect)\textsuperscript{[57, 58]}. This effect on hepatocytes is not only found in cell line experiments and model organisms\textsuperscript{[28, 33, 59-61]} but also discovered in liver organoids from human embryonic stem cells and patient-derived-induced pluripotent stem cells\textsuperscript{[62, 63]}, which may provide a powerful strategy for personalized toxicology evaluation. Furthermore, microfluidic technology has kept evolving in recent years with more efficient approaches for the identification, separation, and quantification of microplastics\textsuperscript{[64]}. Microfluidics is also widely applied to isolation, analysis, and parallel manipulation of single cells\textsuperscript{[65, 66]}. Combining these two research fields with microfluidics may take its advantage of manipulating small volumes of samples within micrometer-scale structures with a point-of-care potential. Lastly, the “long-term uncontrolled inflammation” by MPs/NPs can be a cause of tumor induction. Although one epidemiological study suggested polyvinyl chloride
MPs exposure may increase the risk of liver cancers\textsuperscript{[67]}, it is uncertain whether the carcinogenic effect is caused by microplastics or vinyl chloride monomer \textit{per se}. Nevertheless, the more prominent existence of different MPs in cirrhotic patients than in healthy subjects implies that MPs/NPs may play a more important role in precancerous lesions\textsuperscript{[49]}. More pre-clinical and population-based research evidence is needed to delineate the correlation between MPs/NPs and liver cancers.

**CONCLUSION**

While trying to close the knowledge gap for plastic pollution, scientists are facing some specific challenges. The discrepant results among studies can be owing to various characterizations of MPs/NPs or different experimental protocols. Future experimental designs need to take the type, size, shape, and surface groups of MPs/NPs into consideration. It is also imperative to set exposure concentration and duration comparable to the realistic environment. Standardization of the materials and methods may yield more consistent results. Moreover, the current literature lacks clinical and epidemiological studies. Conducting human population studies can elucidate the association between the MPs/NPs exposure and health outcomes. With advancing analytical technologies, new experimental models, and well-informed interdisciplinary research collaborations, we expect to gain deeper insight into the risk of MPs/NPs to liver health, which will benefit the development of mitigation strategies and policies.
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