Malays J Pathol 2025; 47(2) : 221 – 231

REVIEW ARTICLE

Effect of microplastics and nanoplastics in gastrointestinal tract on gut health: A systematic review

Yi Huan TAN[1], Norfilza MOKHTAR[2,3], Raja Affendi RAJA ALI[3,4] and Lai Ti GEW[1]*

[1]Department of Biomedical Sciences, Sir Jeffrey Cheah Sunway Medical School, Faculty of Medical and Life Sciences, Sunway University, Petaling Jaya, Malaysia; [2]Research Management Centre, Management and Science University, Malaysia; [3]Gut Research Group, Faculty of Medicine, Universiti Kebangsaan Malaysia; [4]Sir Jeffrey Cheah Sunway Medical School, Faculty of Medical and Life Sciences, Sunway University, Petaling Jaya, Malaysia

Abstract

Introduction: Microplastics (MPs) and nanoplastics (NPs) are pervasive environmental contaminants with growing concerns about their ingestion through food and water sources. Although animal studies suggest adverse health effects, direct mechanistic evidence in human gastrointestinal (GI) systems remains limited. *In vitro* models using human GI cell lines and organoids offer a physiologically relevant platform for investigating the effects of MPs and NPs on human health. However, existing findings are fragmented and lack systematic synthesis. This systematic review aims to consolidate and critically analyse current evidence on the biological effects of MPs and NPs in human GI *in vitro* studies. *Materials and Methods:* Articles were selected from a previously conducted systematic search across Scopus and PubMed databases. Studies excluded from the prior review but relevant to MPs and NPs effects on human GI cells were re-screened under newly defined inclusion and exclusion criteria. *Results:* A total of 30 studies were included. MPs and NPs were shown to induce size- and concentration-dependent biological effects, including increased cellular uptake, oxidative stress, mitochondrial dysfunction, inflammation, and apoptosis. Smaller particles consistently exhibited greater cellular internalisation and biological effects. These effects mainly occurred at high concentrations. Under chronic exposure, most studies reported minimal or no significant effects except for cell viability. *Conclusion:* This review provides the first comprehensive synthesis of *in vitro* evidence on the biological effects of MPs and NPs in human GI models. It advances mechanistic understanding and outlines future directions to strengthen health risk assessment, inform strategies for disease prevention, and guide public health policies addressing microplastic exposure.

Keywords: Gastrointestinal tract, plastic pollution, microplastics, nanoplastics, health risks

INTRODUCTION

The global prevalence of microplastics (MPs; <5 mm) and nanoplastics (NPs; <1 μm) in the environment has emerged as a pressing global concern. These synthetic polymer particles, originating from industrial processes, consumer goods, and environmental degradation, have been detected in diverse ecosystems and human consumables, including drinking water, seafood, and table salt.[1] Consequently, the gastrointestinal (GI) tract has been recognised as a primary route of human exposure, warranting urgent investigation into the potential health implications of chronic MPs and NPs ingestion.[1,2]

While environmental toxicology studies have established the bioaccumulation and ecotoxicological effects of MPs and NPs in marine and terrestrial organisms, the extrapolation of these findings to human health remains uncertain.[3] Animal models suggest that MPs and NPs may compromise intestinal barrier function, trigger inflammatory responses, and induce oxidative stress.[4] However, ethical limitations, physiological differences, and complex host-microbiome interactions necessitate the development of alternative approaches that more precisely simulate human-specific responses.

**Address for correspondence:* Department of Biomedical Sciences, Sir Jeffrey Cheah Sunway Medical School, Faculty of Medical and Life Sciences, Sunway University, No. 5, Jalan Universiti, Bandar Sunway, 47500 Petaling Jaya, Malaysia. Tel: +603-79674079 (LTG); Email: *janeg@sunway.edu.my* (LTG)

TABLE 1: Search strings used to perform search in SCOPUS and PubMed (Tan *et al*., unpublished data, 2025)

Search Strings
(microplastic* OR nanoplastic*) AND (human) AND ("digest*" OR "gastrointestinal" OR "alimentary" OR "mouth" OR "oral cavity" OR "pharyn*" OR "throat" OR "oesophag*" OR "esophag*" OR "stomach" OR "intestin*" OR "duoden*" OR "jejun*" OR "ileum" OR "appendi*" OR "cecum" OR "caecum" OR "colon" OR "rectum" OR "rectal" OR "anus" OR "colorectal" OR "anal" OR "bowel" OR "gut" OR "stool" OR "feces" OR "faeces")

In vitro models, particularly human GI cell lines and organoid systems derived from primary tissues have emerged as pivotal tools to bridge this knowledge gap. These models offer a controlled, reproducible environment to examine cellular uptake mechanisms, cytotoxicity pathways, and molecular signalling alterations upon MPs and NPs exposure. Importantly, organoid models recapitulate the cellular diversity and three-dimensional architecture of the human intestine, providing unprecedented opportunities to study complex interactions between MPs and NPs and gut epithelial integrity.[5]

Despite the growing research in *in vitro* studies, the existing evidence remains fragmented. Variability in polymer types, particle sizes, surface charges, exposure concentrations, and experimental designs complicates the ability to draw coherent conclusions about MPs and NPs toxicity. To date, no comprehensive systematic review has synthesised the biological effects of MPs and NPs specifically on human gastrointestinal cell models, critically analysing the mechanistic pathways and contextualising findings across diverse experimental models and conditions.

This systematic review aims to consolidate current evidence on the *in vitro* effects of MPs and NPs on human GI cell lines and organoids. By focusing on key biological endpoints, including cellular uptake, membrane integrity, mitochondrial dysfunction, oxidative stress, DNA damage, cytotoxicity, inflammatory responses, gene expression modulation, and apoptosis, this review provides an integrated framework to better understand the human health risks associated with MPs and NPs exposure. The synthesis also highlights critical research gaps and outlines future directions necessary to advance risk assessment and regulatory decision-making in this rapidly evolving field.

MATERIALS AND METHODS

The articles included in this systematic review were identified through a comprehensive literature search initially conducted for a previous systematic review (Tan *et al*., unpublished data, 2025). The original search was performed across the Scopus and PubMed databases using the search string presented in TABLE 1. The search was executed in July 2023, covering studies published up to that date. It was designed to capture studies investigating the biological effects of MPs and NPs.

During the initial screening process for Tan *et al*. (unpublished data, 2025), several articles were excluded because they did not align with the specific research focus of that review. However, these excluded articles provided valuable data relevant to the present systematic review, which investigates the biological effects of MPs and NPs on human GI cell lines and organoid models.

For the current review, we revisited the pool of retrieved articles from the original search. A new set of inclusion and exclusion criteria was applied to select studies appropriate for the current research objective (TABLE 2). No additional database searches were conducted beyond the original retrieval. As a result, a total of 30 articles were selected and reviewed in the present study (FIG 1).

TABLE 2: Inclusion and exclusion criteria of the reviewed articles

Inclusion criteria	Exclusion criteria
• Studies using human GI cell lines or human intestinal organoid models. • Studies evaluating biological effects of MPs and NPs, including cellular uptake, membrane integrity, oxidative stress, inflammation, apoptosis, or gene expression. • *In vitro* experimental designs.	• Studies focusing solely on non-GI cell lines or animal models. • Studies without experimental biological data (e.g., reviews, editorials). • Studies investigating effects not related to gut epithelial integrity or biological responses.

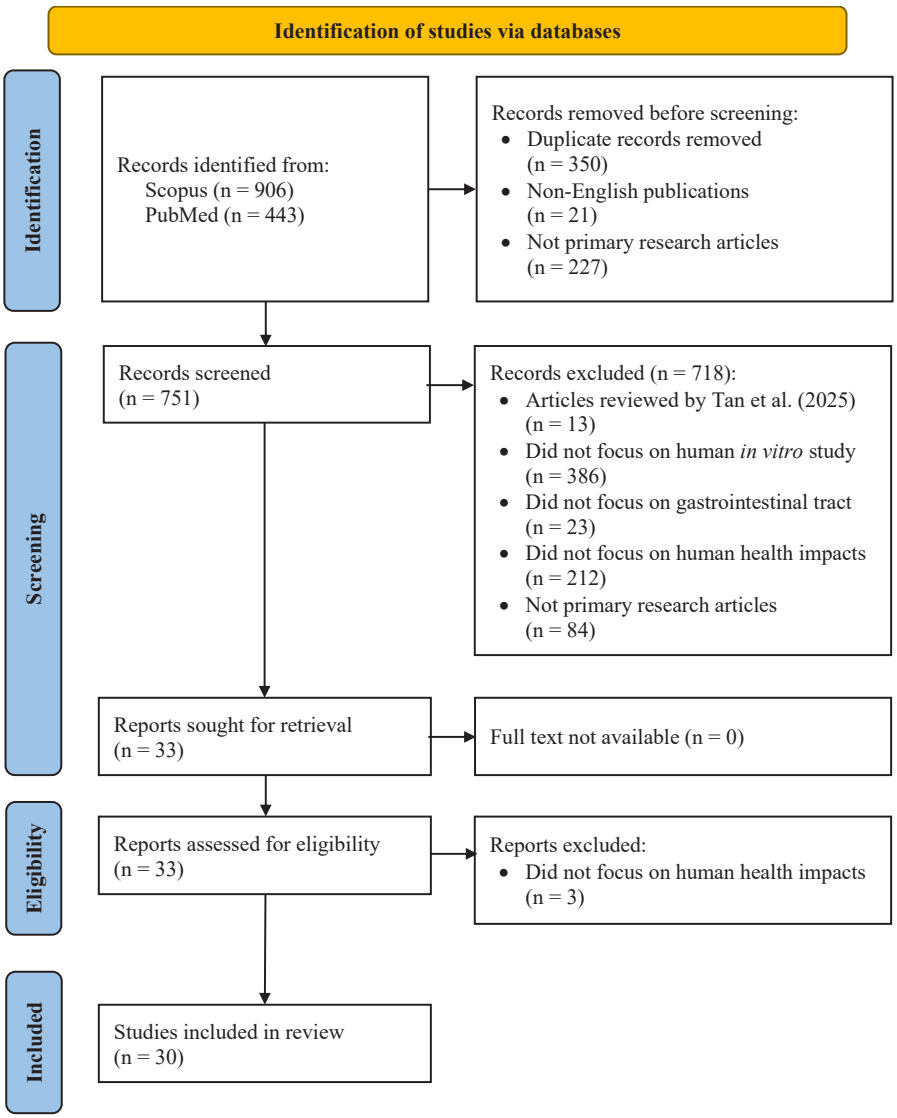

FIG 1. PRISMA flow diagram of the present systematic review.

RESULTS

Type of cell lines and cell models

Cost-effectiveness, practicality, and ethical considerations often favour *in vitro* models. Among the various intestinal cell lines employed, Caco-2 cells emerged as the most popular model as reported in 21 studies.[6–26] Other intestinal cell lines utilised in MPs and NPs *in vitro* studies include HRT-18 as reported by Mattioda *et al*.[27] In addition, comparisons are often made between cell lines representing different parts of the GIT, and between normal and cancerous intestinal cell lines. For example, studies have compared HIEC-6 (small intestine) with CCD841CoN (colon),[28] and Caco-2 (intestinal barrier) and HePG2 (liver).[7] Moreover, in comparison between the biological effect of MPs on normal and cancerous intestinal cell line, Xu *et al*.[29] used one normal intestinal cell line (HIEC-6) and three carcinoma intestinal cell lines (RKO, HT-29 and HCT-116) while Ma *et al*.[18] used NCM460 (normal) and Caco-2 (cancerous) human intestinal epithelial cell lines.

Other than monolayer, co-culture can be developed in MPs and NPs studies as using only one cell type might not give a holistic picture of how MPs and NPs affect the gut immune response. Co-culture allows researchers to investigate more intricate interactions in a simulated gut environment. This can reveal how different cell types collaborate or influence each

other's responses to MPs and NPs, providing a more comprehensive understanding of potential health impacts.[20] In this review, 8 studies established and utilised co-culture models to simulate the human intestinal tissue complex structure and functions.[6,20–25,30] The co-culture models often consist of intestinal epithelial cells (Caco-2 cells), goblet cells for mucus secretion (HT-29, HT29-MTX, or HT29-MTX-E12 cells).[6,20–23,25,30] A more complex tri-culture model can be established by incorporating a third element, Raji B lymphocyte cells, to differentiate Caco-2 cells into microfold cells (M cells).[21,25] M cells are crucial for the transportation of molecules and substances through phagocytosis and transcytosis.[20]

In addition, intestinal organoids are effective cell models being employed for *in vitro* MPs studies. In this review, two studies utilised intestinal organoids for their studies.[5,31] Chen *et al*.[31] derived the intestinal organoid models from isolated tissue biopsies of the human ileum while the model of Hou *et al*.[5] was derived from human induced pluripotent stem cells (hiPSCs). The intestinal organoid established by both studies consisted of absorptive enterocytes, goblet cells, Paneth cells, endocrine cells, and M cells. Enterocytes are dedicated to efficient nutrient and small molecules absorption, while goblet cells contribute to the formation of a protective mucosal barrier through mucus secretion. Enteroendocrine cells play a regulatory role by producing peptides and hormones that influence diverse physiological processes, ensuring gut homeostasis. Paneth cells actively engage in innate immune defence by secreting antimicrobial proteins, thereby maintaining a balanced microbial environment in the intestines. Finally, M cells, with their unique capability for immune sensing and antigen uptake, initiate crucial immune responses within the gut-associated lymphoid tissue. The collaboration of these cells showcases the complexity needed to maintain gut epithelial integrity and overall gastrointestinal health.[31]

Type of plastic polymers exposed to the cell lines

Plastics exist in various polymer types. According to Plastic Europe[32], polyethylene (PE; 26.9%) is the highest type of plastic being produced globally in 2021, followed by polypropylene (PP; 19.3%), polyvinyl chloride (PVC; 12.9%), polyethylene terephthalate (PET; 6.2%), and polystyrene (PS; 5.3%). However, PE was only employed by 5 studies[8–10,22,26] while PS turned out to be the most common type of plastic polymer being used in the cell line studies (n = 22)[5,6,11–18,21,23–25,27–31,33–35]. This is because PS is commercially available with a wide range of specific sizes and shapes, making it convenient for researchers to obtain and use in their studies. PS is relatively inert and has minimal cytotoxicity compared to other plastics. This means it is less likely to interfere with the biological processes of the cells being studied, leading to more reliable results.[31] PP[26], PVC[6,8,26], and PET[7,19,26] are also among the common candidates as model particles with being utilised in up to three studies. While PC may not rank at the forefront of global plastic production, they have captured the attention of researchers due to the widespread presence of these polymers in the food sector where they are commonly used as packaging materials or containers for plastic bottles.[7] Interestingly, Lehner *et al*.[20] utilised the polymer type represent tire wear (rubber) and polyolefins (PP, PA, TPU, softer TPU known as cross-linked polyurethanes), and healing earth that is intended for human consumption. These polymers are commonly used in industries and thus become the major sources of MPs in the Europe.[20]

DISCUSSION

This review focuses on *in vitro* studies, examining the biological effects of MPs and NPs, including their cellular uptake, impact on gastrointestinal cell membrane integrity, mitochondrial activity, oxidative stress, DNA damage, cell viability, cytotoxicity, inflammatory responses, gene expression, and apoptosis. Findings from cell line models form the basis for understanding potential health risks and inform more complex *in vivo* or clinical studies. A summary of the biological effects of MPs and NPs on gastrointestinal cell models is provided in TABLE S1.

Cellular uptake

Upon ingesting food or liquids contaminated with MPs and NPs, these particles may encounter gut epithelial cells. Understanding how MPs and NPs are taken up by cells is crucial, as this determines their potential to exert cytotoxic effects.[16]

MPs and NPs can enter cells through endocytosis,[5,17,19,23,31,33] phagocytosis,[12,14,23] and direct diffusion.[23] Studies have confirmed that particles smaller than 100 nm are internalised by enterocytes and goblet cells via endocytosis.[31] MPs and NPs have been found in lysosomes[16,19,29]

and endosomes[8], further supporting this pathway. In addition, Hou *et al*.[5] delved into the impact of endocytosis on the internalisation of NPs in different cell types in the intestinal organoids, revealing a higher propensity for accumulation in goblet cells, Paneth cells, and endocrine cells compared to enterocyte cells. While enterocytes are primarily responsible for nutrient absorption and particle transport, the unique functions and features of goblet cells, Paneth cells, and endocrine cells might make them more prone to accumulating microparticles and nanoparticles as part of their specialised roles in maintaining gut homeostasis and responding to environmental stimuli.[31] Some larger particles are taken up by M cells through transcytosis.[31]

MPs and NPs have been detected in the cell membrane[34], cytoplasm[13,23,34], lysosome[16,29], and nuclei.[13,15,21,23] In Caco-2 cells, most NPs were near the membrane, while some MPs were freely in the cytoplasm, where they can disrupt organelles and cause oxidative stress.[17,23,34] MPs in lysosomes may lead to membrane rupture and apoptosis.[16] Internalisation of MPs and NPs in the lysosomes was also reported in not only normal intestinal epithelial cell line (HIEC-6) but also colon carcinoma cell lines (RKO, HT-29, and HCT-116).[29] MPs and NPs in nuclei raise concerns about DNA damage and mutations, especially as they have been observed interacting with chromosomes.[13]

A consistent observation across studies is the strong influence of particle size on MPs and NPs uptake and internalisation. Smaller particles exhibit a higher propensity for cellular uptake compared to larger MPs.[16,17,25,26] In line with that, this size-dependent trend is evident in Caco-2 cells, where MPs showed a slower uptake rate compared to NPs.[12,17,18] Notably, studies show NPs smaller than 500 nm have uptake rates over 70%, while larger MPs (1–6 μm) have reduced uptake rates (30–49%).[17] As the size-dependent effect was not cell type specific, this size-dependent effect was consistent across various cell lines, including CCD-18Co[33], HIEC-6[28,29],CCD841CoN[28], SNU-1[34], and NCM460.[18] Remarkably, HIEC-6 cells showed a higher uptake of MPs and NPs than CCD841CoN cells, indicating a greater uptake capacity in HIEC-6.[28]

In intestinal organoid models, a consistent negative correlation between particle size and uptake rate was demonstrated, with higher uptake for smaller NPs (30 nm) compared to MPs.[31] The co-culture model further affirmed this trend, showing the highest uptake and translocation for 25 nm NPs, while the least was observed for 1000 nm MPs.[23]

Membrane integrity

Cell membranes serve as the first-line barrier for MPs and NPs seeking entry into cells and exerting biological effects. The uptake of MPs and NPs through the membrane causes the cell membrane to lose its stability. Membrane integrity may be compromised by this interaction, potentially affecting cellular functions.

However, membrane integrity was not affected in Caco-2 cells, as reported in the majority of studies (3 out 4), where no significant lactate dehydrogenase (LDH) leakage was detected.[16,19,23] LDH is an enzyme inside cells that leaks out when the cell membrane is damaged. Measuring its concentration in the surrounding medium helps to assess the membrane integrity. In addition, no changes of trans-epithelial electrical resistance (TEER) values was also reported in co-culture models[6,21–23,30] except for one study that reported slight barrier integrity and permeability impairment by 20 nm NPs at high concentration (more than 100 μg/mL).[24] TEER measures the resistance of epithelial layers to ion passage, indicating barrier integrity. A high TEER means a tight, intact barrier, while a decrease suggests damage, increased permeability and potential MP translocation.

In addition, tight junctions play a pivotal role in maintaining the integrity and permeability of the intestinal barrier by selectively controlling the passage of substances across the intestinal barrier.[20] Cui *et al*.[24] reported downregulated mRNA and protein expression levels of the tight junction proteins, indicating the impairment of barrier integrity and permeability.

Remarkably, several studies reported size-dependent effect on intestinal membrane integrity.[12,31] Using Caco-2 cell lines, Saenen *et al*.[12] compared the effect on cell membrane integrity of MPs and NPs in different sizes i.e. 2 μm and 200 nm, respectively. Higher uptake was observed for the smaller 200 nm particles compared to the bigger 2 μm particles. In addition, Chen *et al*.[31] studied the effects of plastic particles (1 μm, 500 nm, 100 nm, and 30 nm) on human intestinal organoids. TEER values remained stable with 1 μm, 500 nm, and 100 nm MPs, showing little to no barrier damage. However, 30 nm NPs caused significant damage at very high concentrations (1000 μg/mL).

Mitochondrial activity

Mitochondrial membrane potential (MPP) plays a pivotal role in cellular function, serving as an indicator of mitochondrial health and functionality. The mitochondria are crucial organelles responsible for energy production and cellular metabolism. Their proper functioning hinges on maintaining a stable MPP, a voltage gradient across the inner mitochondrial membrane essential for adenosine triphosphate (ATP) synthesis and other critical processes. Disruption of this potential can lead to a cascade of detrimental effects, impacting cell viability, function, and ultimately, survival.[28]

Studies across various cell lines revealed a complex interplay between MPs/NPs and MPP. In Caco-2 intestinal epithelial cells, Cortés *et al*.[15] observed elevated MPP, suggesting the induction of mitochondrial stress. Similarly, Saenen *et al*.[12] reported a decrease in the intracellular H_2O_2 levels which led to an increase of the MPP, inducing mitochondrial stress responses. In contrast, Wu *et al*.[16] reported a decrease in MPP due to mitochondrial depolarisation. It is noteworthy mitochondrial depolarisation is observed to be size dependent. Specifically, 5 μm MPs triggered more significant mitochondrial depolarisation compared to 0.1 μm NPs, implying a decrease in MPP.

Similar reductions in MPP were observed in HIEC-6 and CCD841CoN cells, linked to mitochondrial electron transport chain impairment.[28] Notably, HePG2 cells showed even greater sensitivity to MPs and NPs than Caco-2 cells.[7] Fluctuations in MPP, whether increasing or decreasing, can disrupt energy production and cell survival, highlighting the need for mitochondrial stability.

Slight fluctuations in MPP might be tolerated and even trigger adaptive responses, but significant deviations can have detrimental consequences, impacting cellular energy production, signalling pathways, and ultimately cell survival and organ function.[36]

Oxidative stress

The assessment of oxidative stress is marked by an imbalance between reactive oxygen species (ROS) production and the cell's antioxidant defense mechanisms. Oxidative stress can arise when exposure to plastic particles leads to an overproduction of ROS, potentially causing cellular damage and various adverse effects such as cellular apoptosis.[9,28]

In gastrointestinal monolayer cell models like Caco-2, HRT-18, and NCM460, no significant ROS production was observed.[9,15,18,19,27] Similarly, in co-culture models, two studies assessed oxidative stress from MPs and NPs exposure and found no significant ROS production, indicating no oxidative stress.[21,23] The absence of oxidative stress can be further analysed and confirmed by the changes in the expression of ROS-related genes such as *HO1*, *SOD2*, and *GSTP1* genes. However, inconsistent results were demonstrated by Wu *et al*.[16] using Caco-2. MPs and NPs were reported to significantly increase intracellular ROS generation, leading to the induction of oxidative stress.[5,16]

The relationship between exposure time and ROS level also reveals intriguing dynamics. MPs can induce ROS production in HT-29 cells in both acute (up to 24 hours) and sub-chronic (up to 48 days) exposure.[9,35] Studies found ROS levels peaked at 6 hours but started decreasing after 24 hours, with further reduction after 7 days, suggesting HT-29 cells can partially neutralise oxidative stress over time.[9,18,35] Similar trends were seen in CCD-18Co and Caco-2 cells, where prolonged exposure led to a temporary increase in ROS, followed by stabilisation due to antioxidant enzyme activity.[13,33] ROS upregulation, in turn, stimulates the production of antioxidant enzymes, which diligently scavenge these ROS radicals, restoring redox equilibrium and consequently normalizing ROS levels over time.[28] This indicates a strong correlation between exposure duration and ROS regulation.[35]

Particle size also appeared to play a crucial role in the effect on oxidative stress induced by MPs and NPs. Smaller particle seem to be more potent inducers of ROS, potentially due to their enhanced cellular uptake and interaction with organelles.[28,33] Studies found that 0.5 μm NPs caused more oxidative stress than 2 μm MPs in CCD-18Co cells[33], while 0.1 μm particles triggered more ROS than larger particles (0.5–5 μm) in HIEC-6 cells.[28] Similarly, 6.2 μm MPs increased ROS in Caco-2 cells, whereas larger (30.5 μm) MPs showed no significant effect.[10]

DNA damage

DNA holds the genetic code essential for cell function and organismal development. Its integrity is paramount for maintaining cellular health and preventing disease. When MPs is internalised in nuclei, damage to DNA can occur through various mechanisms, including errors during replication, exposure to ionizing radiation, and chemical alterations by ROS. These

disruptions can lead to mutations, chromosomal abnormalities, and ultimately, cell death or the development of diseases like cancer.

DNA damage can be divided into genotoxic DNA damage and oxidative DNA damage.[21] Genotoxic damage involves direct physical alterations to the DNA structure, such as strand breaks or crosslinks.[15] On the other hand, oxidative DNA damage arises from the interaction of ROS with DNA, leading to oxidative lesions.[15]

Studies on Caco-2 cells found no significant genotoxic or oxidative DNA damage from MPs/NPs exposure, even at high concentrations. Similar results were seen in co-culture models, confirming that MPs/NPs do not significantly affect DNA integrity. The absence of DNA damage aligns with the lack of ROS production in these studies.

However, HT-29 cells showed DNA damage after both short-term (24 hours) and prolonged (48 days) MPs exposure. Acute exposure caused DNA fragmentation, but this effect diminished after 28 days, likely due to oxidative stress. These findings suggest that while MPs may induce temporary DNA damage, their long-term impact remains limited.

Despite Caco-2 cells are able to uptake and internalise MPs and NPs in the cell nuclei, studies[13,15] reported no significant genotoxic DNA damage and only slight oxidative DNA damage in response to MPs/NPs exposure, even at high concentrations. Thus, chromosomal damage was also not significant in Caco-2 cells. Similar results were seen in co-culture models, confirming that MPs/NPs do not significantly affect DNA integrity.[6,21,22,30] The absence of DNA damage aligns with the lack of ROS production in these studies.[21] However, HT-29 cells showed DNA damage after both short-term (24 hours) and prolonged (48 days) MPs exposure.[35] Acute exposure caused DNA fragmentation, but this effect diminished after 28 days, likely due to oxidative stress.[35]

Cell viability and cytotoxicity

Cell viability is crucial in understanding the potential harm of MPs and NPs because it provides a direct measure of their cytotoxic effects, revealing their ability to disrupt the fundamental health and function of cells. Cytotoxicity is often linked to membrane damage and oxidative stress. Loss of membrane integrity allows harmful substances to enter, triggering ROS production, which damages mitochondria and leads to cell death. These intricate mechanisms likely contribute to the observed decrease in cell viability following MPs and NPs exposure.[6,27]

Studies on Caco-2[10,12,13,16,19], CCD-18Co[33], NCM460[18], and CCD841CoN[28] cells found no significant cytotoxicity from MPs exposure, regardless of polymer type, size, or concentration. Similarly, co-culture models showed no major impact on cell viability.[6,20,21] However, several studies reported significant cytotoxicity in Caco-2, HT-29, HRT-18, and co-culture models.[9,15,22,27] PE can significantly increase the cytotoxicity up to 70% in Caco-2 and HT-29.[9] Similarly, Busch *et al*.[22] documented significant cytotoxicity in co-culture model at the highest tested exposure concentration (50 µg/mL). Interestingly, in the organoid-derived cell monolayer, the highest testing dose (200 µg/mL) was able to induce only mild cytotoxic effect, with a cell viability loss of up to 29.96%.[5]

Increased cytotoxicity may cause a significant reduction in cell viability as reported by Wang *et al*.[17] and Tolardo *et al*.[7] Significant decrease in cell viability was observed to be concentration dependent. As the exposure concentration increased, MPs caused a linear decrease in cell viability,[17] with reductions of up to 10% at 100 µg/mL,[11] 30% at 1000 µg/mL[9] and 60% at 75,000 µg/mL[26] in extreme cases.

Exposure time also significantly affects the impact of MPs and NPs on cell viability. Short-term (24-hour) exposure increased viability in Caco-2, RKO, HT-29, and HCT-116 cells, but a notable decline was observed after 48 hours at high concentration.[9,11,35]

Furthermore, the impact on cell viability and cytotoxicity was found to be size-dependent, with smaller particles exhibiting a more pronounced effect on cell loss.[16,24,25,34] Hence, this explains the higher observed cytotoxicity of NPs smaller than 50 nm.[16,24,34] In contrast, larger particles (100 nm - 5 µm) had little to no cytotoxic effect.[16,24,25,34] Smaller particles were more toxic due to easier cell entry and accumulation.

Inflammatory responses

Inflammatory responses play a key role in the body's defense against foreign invaders like MPs and NPs. However, chronic or uncontrolled inflammation can be detrimental, leading to tissue damage and various diseases. Several pro-inflammatory cytokines are commonly measured in MPs/NPs cell line studies, including interleukin-1β,6,8 (IL-1β,6,8) and

tumour necrosis factor-α (TNF-α). IL-8 plays a crucial role in attracting neutrophils and other immune cells to the site of inflammation, amplifying the inflammatory responses. IL-1β is an early-stage pro-inflammatory cytokine. Upregulation of IL-1β initiates and amplifies the inflammatory cascade by activating other immune cells and inducing further cytokine production. TNFα serves as a powerful pro-inflammatory and immunomodulatory cytokine that activates immune cells, promotes tissue damage during inflammation, and regulates cell survival and apoptosis. IL-6 is important in acute inflammation. It stimulates B cell antibody production to induce the acute-phase inflammatory response and promote the differentiation of immune cells.

Several studies[10,19,20] reported no significant alterations in pro-inflammatory cytokine levels, even after long-term MPs exposure for up to 8 weeks.[13] However, one study demonstrated a clear pro-inflammatory response with increased IL-8 expression when treated with PS MPs.[27] Interestingly, the type of polymer seems to play a crucial role, with PS showing minimal impact while PVC induced a two-fold increase in IL-1β.[6] This highlights the importance of considering not only the presence of MPs and NPs but also their material composition and physicochemical properties when assessing their inflammatory potential.

Intestinal organoid studies showed that smaller NPs (100 nm, 30 nm), at high concentrations (500–1000 μg/mL), significantly increased TNF-α, IL-6, and IL-8 secretion, suggesting that both size and concentration influence inflammatory effects.[31] Hou *et al*.[5] further supported this notion by demonstrating significantly elevated NF-κB p65 and IL-8 levels in intestinal organoids exposed to 50 nm NPs. NF-κB is a key regulator of inflammatory responses, and its activation by NPs suggests a potential mechanism for the observed increase IL-8 secretion. Additionally, Hou *et al*.[5] also reported upregulation of α-defensin-5, a biomarker linked for CD.[37] Therefore, the upregulation of α-defensin-5 in intestinal organoids exposed to NPs suggests a potential link between MPs/NPs exposure and the development of Crohn's colitis-like inflammation. Observing this association *in vitro* raises concerns about the possible long-term consequences of chronic exposure to MPs and NPs *in vivo*, contributing to the development or even exacerbating pathophysiological processes in the gastrointestinal tract, such as chronic inflammatory states and gastric and colonic carcinomas.[27]

Gene expression

Gene expression is often used as a confirm test to validate the inflammatory responses and oxidative stress induced by MPs and NPs.[13,15,21,27] Upregulation of genes encoding pro-inflammatory cytokines like IL-1β, IL-6, and TNF-α indicates the activation of inflammatory pathways. In addition, overexpression of genes for antioxidant enzymes such as *HO1*, *SOD2*, and *GSTP1* indicates the cell's attempt to counteract the increased generation of ROS induced by the MPs and NPs.

HRT-18 cells showed a significant increase in *IL-8* gene expression, which is line with the inflammation observed in the study.[27] Other inflammation-related genes such as *TRPV1*, *iNOS*, *IL-1β* were also reported to be up-regulated.[11] In contrast, neither the expression of *IL-1β* nor that of *IL-8* showed significant changes in Caco-2 cells.[15]

To further confirm the oxidative stress responses, the expression of stress-related genes were examined.[13,15,21] The common candidate genes for oxidative stress are *HO1*, *SOD2*, and *GSTP1*. While some studies observed a slight tendency towards increased expression of ROS-related genes in Caco-2 cells, these changes often lacked statistical significance.[13,15,21] Only *HO1* was significantly upregulated. The *HO1* gene encodes the enzyme heme-oxygenase-1 (HO1), which catabolises heme into biliverdin, carbon monoxide, and free iron. Biliverdin is further converted into bilirubin, a potent antioxidant with anti-inflammatory properties which may respond to various stimuli, including hypoxia and oxidative stress.[13] Chronic exposure to MPs and NPs reveals a different picture. Domenech *et al*.[13] demonstrated significant overexpression of *HO1* and *SOD2* in Caco-2 cells exposed to NPs for eight weeks. The *SOD2* gene encodes the enzyme mitochondrial manganese superoxide dismutase (SOD2), which scavenges superoxide radicals, a highly reactive type of ROS, that originates from oxidative phosphorylation. SOD2 is localised in the mitochondrial matrix, where it plays a critical role in protecting mitochondrial DNA and proteins from oxidative damage.[13] This indicates a sustained stress response, suggesting potential long-term consequences associated with chronic MPs and NPs exposure. Interestingly, other stress-related genes like *HSP70* and *GSTP1* showed no significant changes in either short-

term or long-term exposure.[13,15,21] This suggests a selective activation of specific stress response pathways, highlighting the complexity of cellular responses to MPs and NPs.

Apoptosis

Apoptosis is a controlled, programmed process of cell suicide, minimising damage to surrounding tissues and triggering efficient clearance of dead cells. Apoptosis can be triggered by various cellular stresses, including DNA damage, oxidative stress, and mitochondrial dysfunction.[28]

Studies involving Caco-2 cells showed consistent results. Magrì *et al*.[19] and Busch *et al*.[6] did not show any significant change in apoptosis compared to the control group. A similar result was demonstrated in mucus-secreting goblet cells, HT29-MTX-E12, at the highest testing concentration (50 μg/cm^2).[6] However, the intestinal organoid model[5] showed a significant increase in apoptosis level.

Examining the impact of different sizes of MPs and NPs on apoptosis, Banerjee *et al*.[34] reported size-dependent apoptotic effect in SNU-1 gastric cells, with the greatest apoptotic effect caused by the smallest 50 nm NPs. This could be linked to the intense cellular uptake of NPs, inducing high cellular stress and apoptosis in a majority of the cells.[34]

Xu *et al*.[29] utilised both normal (HIEC-6) and cancerous (RKO, HT-29, and HCT-116) gastrointestinal cell lines to study the apoptotic effect of MPs and NPs. In both types of intestinal cell lines, Xu *et al*.[29] reported a dose-dependent increase in Annexin V-positive cells, indicating early apoptosis induction. Annexin V is a protein that binds to phospholipids on the surface of cells undergoing apoptosis or programmed cell death. This makes it a marker for identifying dying or damaged cells. Remarkably, the normal intestinal cell line exhibited a relatively stronger apoptotic effect than the cancerous one.[29] Zhang *et al*.,[28] on the other hand, found significantly higher apoptotic rate in small intestine (HIEC-6) than colon cell lines (CCD841CoN), though the overall apoptotic level remained low (<8%).

The observed differential susceptibility to apoptosis across cell lines suggests possible variations in cellular defense mechanisms and apoptotic pathways. The apparent resistance of colon carcinoma cells compared to normal intestinal epithelial cells may be related to their altered genetic and signalling profiles. Further research investigating these differences could provide valuable insights into the specific mechanisms by which MPs and NPs trigger apoptosis. While the underlying mechanisms by which MPs and NPs induce apoptosis remain unclear. Zhang *et al*.[28] suggested that MPs might interact with the cell membrane, disrupting the balance of the ETC. This disruption could lead to a decline of the MPP and potentially trigger early apoptosis.

CONCLUSION

This systematic review critically evaluated the biological interactions between MPs and NPs and human GI cell models, synthesising insights from numerous *in vitro* studies. The evidence consistently demonstrates that particle size, polymer type, exposure concentration, and model variation are major determinants of cellular responses to MPs and NPs. Overall, smaller particles consistently demonstrated greater cellular internalisation and induced greater biological effects, including oxidative stress, inflammatory responses, mitochondrial dysfunction, and apoptosis. However, these effects were predominantly observed at high exposure concentrations, often exceeding expected environmental levels. Under chronic exposure conditions, most studies reported minimal or no significant adverse effects, aside from cell viability.

The present synthesis exposes critical gaps in the field, including the need for standardised particle characterisation, harmonised exposure protocols, and integration of gut microbiota interactions. The heterogeneity in experimental designs currently limits meta-analytical approaches and hinders the translation of findings into quantitative risk assessments.

Moving forward, multidisciplinary efforts are essential to bridge *in vitro* observations with *in vivo* outcomes and epidemiological data. Research focusing on chronic, low-dose exposures, degraded particles, and immunologically competent models will be pivotal. Furthermore, systematic investigation into polymer-specific toxicities and cumulative effects is imperative to guide evidence-based regulatory frameworks and public health strategies. By consolidating mechanistic evidence, this review provides a critical foundation for advancing the understanding of the effects of MPs and NPs in human health.

Acknowledgements: YH Tan would like to thank Sunway University for PhD Studentship from Sunway University, Malaysia. The authors would

like to thank Sunway University Malaysia for continuous support in research. This research was funded by Fundamental Research Grant Scheme (Grant no.: FRGS/1/2023/SKK10/SYUC/01/1) awarded by Ministry of Higher Education (MOHE) Malaysia.

Informed Consent Statement: Not applicable.

Author' contributions: Conceptualization, LTG, RARA; formal analysis, YHT, LTG, RARA; writing, YHT; review and editing, LTG, RARA, NM; supervision, LTG, RARA, NM; final approval of the manuscript, LTG, RARA, NM. All authors have read and agreed to the published version of the manuscript.

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

REFERENCES

1. Jin M, Wang X, Ren T, Wang J, Shan J. Microplastics contamination in food and beverages: Direct exposure to humans. J Food Sci. 2021;86(7):2816–2837.
2. OECD. Global Plastics Outlook: Economic Drivers, Environmental Impacts and Policy Options. 2022.
3. van Raamsdonk LWD, van der Zande M, Koelmans AA, *et al*. Current insights into monitoring, bioaccumulation, and potential health effects of microplastics present in the food chain. Foods (Basel). 2020;9(1):72.
4. Huang Z, Weng Y, Shen Q, Zhao Y, Jin Y. Microplastic: A potential threat to human and animal health by interfering with the intestinal barrier function and changing the intestinal microenvironment. Sci Total Environ. 2021;785:147365.
5. Hou Z, Meng R, Chen G, *et al*. Distinct accumulation of nanoplastics in human intestinal organoids. Sci Total Environ. 2022;838:155811.
6. Busch M, Bredeck G, Kämpfer AAMM, Schins RPFF. Investigations of acute effects of polystyrene and polyvinyl chloride micro- and nanoplastics in an advanced in vitro triple culture model of the healthy and inflamed intestine. Environ Res. 2021;193:110536.
7. Tolardo V, Magrì D, Fumagalli F, *et al*. In vitro high-throughput toxicological assessment of nanoplastics. Nanomaterials (Basel). 2022;12(12):1947. doi:10.3390/nano12121947
8. Cassano D, Bogni A, La Spina R, Gilliland D, Ponti J. Investigating the cellular uptake of model nanoplastics by single-cell ICP-MS. Nanomaterials. 2023;13(3):594.
9. Herrala M, Huovinen M, Järvelä E, *et al*. Micro-sized polyethylene particles affect cell viability and oxidative stress responses in human colorectal adenocarcinoma Caco-2 and HT-29 cells. Sci Total Environ. 2023;867:161512.
10. Gautam R, Jo JH, Acharya M, *et al*. Evaluation of potential toxicity of polyethylene microplastics on human derived cell lines. Sci Total Environ. 2022;838:156089.
11. Wu S, Wu M, Tian D, Qiu L, Li T. Effects of polystyrene microbeads on cytotoxicity and transcriptomic profiles in human Caco-2 cells. Environ Toxicol. 2020;35(4):495–506.
12. Saenen ND, Witters MS, Hantoro I, *et al*. Polystyrene microplastics of varying sizes and shapes induce distinct redox and mitochondrial stress responses in a Caco-2 monolayer. Antioxidants. 2023;12(3):739.
13. Domenech J, de Britto M, Velázquez A, *et al*. Long-term effects of polystyrene nanoplastics in human intestinal Caco-2 cells. Biomolecules. 2021;11(10):142.
14. Zhang YX, Wang M, Yang L, Pan K, Miao AJ. Bioaccumulation of differently-sized polystyrene nanoplastics by human lung and intestine cells. J Hazard Mater. 2022;439:129585.
15. Cortés C, Domenech J, Salazar M, Pastor S, Marcos R, Hernández A. Nanoplastics as a potential environmental health factor: Effects of polystyrene nanoparticles on human intestinal epithelial Caco-2 cells. Environ Sci Nano. 2019;6(7):1692–1704.
16. Wu B, Wu X, Liu S, Wang Z, Chen L. Size-dependent effects of polystyrene microplastics on cytotoxicity and efflux pump inhibition in human Caco-2 cells. Chemosphere. 2019;221:333–341.
17. Wang Q, Bai J, Ning B, *et al*. Effects of bisphenol A and nanoscale and microscale polystyrene plastic exposure on particle uptake and toxicity in human Caco-2 cells. Chemosphere. 2020;254:126788.
18. Ma Y, Koh JYC, Lim HK, Shi P, Tay CY. Elucidating the size-dependency of in vitro digested polystyrene microplastics on human intestinal cells health and function. Macromol Chem Phys. 2022;223(13):2100454.
19. Magrì D, Sánchez-Moreno P, Caputo G, *et al*. Laser ablation as a versatile tool to mimic polyethylene terephthalate nanoplastic pollutants: Characterization and toxicology assessment. ACS Nano. 2018;12(8):7690–7700.
20. Lehner R, Wohlleben W, Septiadi D, Landsiedel R, Petri-Fink A, Rothen-Rutishauser B. A novel 3D intestine barrier model to study the immune response upon exposure to microplastics. Arch Toxicol. 2020;94(7):2463–2479.
21. Domenech J, Hernández A, Rubio L, Marcos R, Cortés C. Interactions of polystyrene nanoplastics with in vitro models of the human intestinal barrier. Arch Toxicol. 2020;94(9):2997–3012.
22. Busch M, Kämpfer AAM, Schins RPF. An inverted in vitro triple culture model of the healthy and inflamed intestine: Adverse effects of polyethylene particles. Chemosphere. 2021;284:131345.
23. DeLoid GM, Cao X, Bitounis D, *et al*. 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.
24. Cui M, He Q, Wang Z, *et al*. Mucin2 regulated by Ho1/p38/IL-10 axis plays a protective role

in polystyrene nanoplastics-mediated intestinal toxicity. Environ Pollut. 2023;330:121808.
25. Stock V, Böhmert L, Lisicki E, *et al*. Uptake and effects of orally ingested polystyrene microplastic particles in vitro and in vivo. Arch Toxicol. 2019;93(7):1817–1833.
26. Stock V, Laurisch C, Franke J, *et al*. Uptake and cellular effects of PE, PP, PET and PVC microplastic particles. *Toxicol Vitr*. 2021;70(June 2020):105021.
27. Mattioda V, Benedetti V, Tessarolo C, *et al*. Pro-inflammatory and cytotoxic effects of polystyrene microplastics on human and murine intestinal cell lines. Biomolecules. 2023;13(1):140.
28. Zhang Y, Wang S, Olga V, *et al*. The potential effects of microplastic pollution on human digestive tract cells. Chemosphere. 2022;291(Pt 1):132714.
29. Xu X, Feng Y, Han C, *et al*. Autophagic response of intestinal epithelial cells exposed to polystyrene nanoplastics. Environ Toxicol. 2023;38(1):205–215.
30. Hesler M, Aengenheister L, Ellinger B, *et al*. Multi-endpoint toxicological assessment of polystyrene nano- and microparticles in different biological models in vitro. Toxicol In Vitro. 2019;61:104610.
31. Chen Y, Williams AM, Gordon EB, *et al*. Biological effects of polystyrene micro- and nano-plastics on human intestinal organoid-derived epithelial tissue models without and with M cells. Nanomedicine (Lond). 2023;50:102680.
32. Plastics Europe. Plastics – the Facts 2022. 2022. Available from: https://plasticseurope.org/knowledge-hub/plastics-the-facts-2022/ (accessed 18 January 2023).
33. Bonanomi M, Salmistraro N, Porro D, Pinsino A, Colangelo AM, Gaglio D. Polystyrene micro and nano-particles induce metabolic rewiring in normal human colon cells: A risk factor for human health. Chemosphere. 2022;303:134947. doi:10.1016/j.chemosphere.2022.134947.
34. Banerjee A, Billey LO, Shelver WL. Uptake and toxicity of polystyrene micro/nanoplastics in gastric cells: Effects of particle size and surface functionalization. PLoS One. 2021;16(12):e0260803.
35. Visalli G, Facciolà A, Ciarello MP, De Marco G, Maisano M, Di Pietro A. Acute and sub-chronic effects of microplastics (3 and 10 μm) on the human intestinal cells HT-29. Int J Environ Res Public Health. 2021;18(11):5833.
36. Zorova LD, Popkov VA, Plotnikov EY, *et al*. Mitochondrial membrane potential. Anal Biochem. 2018;552:50.
37. Williams AD, Korolkova OY, Sakwe AM, *et al*. Human alpha defensin 5 is a candidate biomarker to delineate inflammatory bowel disease. PLoS One. 2017;12(8):e0179710. doi:10.1371/journal.pone.0179710.

APPENDICES

TABLE S1. Biological effects of microplastics and nanoplastics in human gastrointestinal cell lines and models.

Reference	Biological models	Polymer	Size	Biological effect	Important Findings
[6]	Caco-2, HT29-MTX-E12 & tri-culture model (Caco-2/HT29-MTX-E12/THP-1)	PS & PVC	50 nm (PS), and <50 µm (PVC)	DNA damage	No significant effect.
				Cell viability	No significant effect.
				Inflammatory response	Significant dose-dependent increased expression of IL-8. PVC > PS
				Apoptosis	Caco-2 & HT29-MTX-E12 No significant effect. HT29-MTX-E12 & tri-culture model (Caco-2/HT29-MTX-E12/THP-1) Significant increased.
[7]	Caco-2 & HePG2	PC & PET	NR	Membrane integrity	PC > PET
				Mitochondrial activity	Significant reduction in mitochondrial activity PC > PET HePG2 > Caco-2 Laser ablation > nanoprecipitation
				Cell viability	Reduced cell viability and increased cytotoxicity. PC > PET
[8]	Caoc-2	PE & PVC	< 1 µm	Uptake	Endocytosis: NPs were mainly distributed inside endosomes with amphisomes.
[9]	Caco-2 & HT-29	PE	5 to 60 µm	Oxidative stress	Induced oxidative stress Time dependent: prolonged exposure induced oxidative stress
				Cell Viability	Concentration dependent: decreased cell viability and increased cytotoxicity at high concentration (1000 ug/mL)

Reference	Biological models	Polymer	Size	Biological effect	Important Findings
[10]	Caco-2	PE	6.2 and 30.5 μm	Oxidative stress	Significantly increased in the level of oxidative stress in 6.2 μm
				Cell viability	No significant effect.
				Inflammatory response	No significant effect.
[11]	Caco-2	PS	NR	Cell viability	No significant effect. Time dependent: acute exposure (24h) increased cell viability while prolonged exposure (48h) decreased cell viability. Concentration dependent: most obvious effect at 100 μg/mL.
				Gene expression	Four inflammation related genes (*TRPV1, iNOS, IL-1β, IL-8*) were up-regulated.
[12]	Caco-2	PS	200 nm and 2 μm	Uptake	Phagocytosis Size dependent: greater uptake of smaller 200 nm NPs.
				Membrane integrity	Size dependent: mainly affected by the 2 μm MPs
				MPP	Induced mitochondrial stress responses.
				Cell viability	No significant effect.
[13]	Caco-2	PS	50 nm	Uptake	NPs are found in the cell cytoplasm and nuclei.
				Oxidative stress	No significant effect.
				DNA damage	Low level of genotoxic and oxidative DNA damage.
				Cell viability	No significant effect.
				Gene expression	No significant effect. Long-term exposure (8 weeks) induced *HO1* and *SOD2* expression.
[14]	Caco-2	PS	70 nm, 200 nm, and 500 nm	Uptake	70 nm – phagocytosis, endocytosis 200 and 500 nm – phagocytosis
				Cell viability	Size dependent: 70 nm > 200 and 500 nm

Reference	Biological models	Polymer	Size	Biological effect	Important Findings
[15]	Caco-2	PS	0.05 to 0.1 μm	Uptake	NPs are found inside the cells.
				MPP	Significantly increase mitochondrial membrane potential.
				ROS	No significant effect.
				DNA damage	No significant effect.
				Cell viability	No significant effect until exposure concentration of 150 μg/mL.
				Gene expression	No significant effect.
[16]	Caco-2	PS	0.1 μm and 5 μm	Uptake	MPs are found in lysosomes, suggesting internalization via endocytosis. Size dependent: 0.1 μm NPs > 5 μm MPs
				Membrane integrity	No significant effect.
				ROS	Increase ROS generation and induce oxidative stress
				Cell viability	No significant effect. Size-dependent: 0.1 μm NPs > 5 μm MPs
[17]	Caco-2	PS	300 nm, 500 nm, 1 μm, 3 μm, and 6 μm	Uptake	Endocytosis Size dependent: The smaller the particle size is, the greater the number of particles that enter cells.
				Cell viability	Increase cytotoxicity and reduce cell viability. Concentration dependent: cell viability decreases as the exposure concentration increased.
[18]	Caco-2 & NCM460	PS	0.1 μm, 1 μm, and 10 μm	Uptake	Smaller particle showed higher uptake rate.
				Oxidative stress	No significant effect Significant increase ROS level in NCM460 at 24 h of exposure time
				Cell viability	No significant effect

Reference	Biological models	Polymer	Size	Biological effect	Important Findings
[19]	Caco-2	PET	100 nm	Uptake	NPs are found in lysosomes, suggesting internalization via endocytosis.
				Membrane integrity	No significant effect.
				Oxidative stress	No significant effect.
				Cell viability	No significant effect.
				Inflammatory response	No significant effect on the production profile of pro-inflammatory cytokine proteins IL-8 and MCP-1.
				Apoptosis	No significant effect.
[20]	Co-culture (Caco-2, HT29-MTX-E12, human blood monocyte-derived macrophages (MDM), human blood monocyte-derived dendritic cells (MDDC))	Tire wear, polyolefins, healing earths	50 to 500 μm	Cell viability	No significant effect.
				Inflammatory response	No significant effect.
[21]	Co-culture (Caco-2 cell/HT29/Raji B cells)	PS	0.05 to 0.1 μm	Uptake	NPs are found in the nuclei of Raji B cells.
				Membrane integrity	No significant effect.
				Oxidative stress	No significant effect.
				DNA damage	No significant effect.
				Cell viability	No significant effect.
				Gene expression	No significant effect in the expression levels of ROS-related gene (*HO1, GSTP1, SOD*) and cellular stress induction gene (*HSP70*)

Reference	Biological models	Polymer	Size	Biological effect	Important Findings
[22]	Tri-culture model (Caco-2/HT29-MTX-E12/THP-1)	PE	200 to 9900 nm	Membrane integrity	No significant effect
				DNA damage	No significant effect
				Cell viability	Significantly decreased cell viability and increased cytotoxicity
				Inflammatory response	Significant dose-dependent increased expression of IL-8
[23]	Tri-culture small intestinal epithelium (Caco-2/HT29-MTX/M-cells)	PS	25 to 1000 nm	Uptake	Direct diffusion Endocytosis Phagocytosis MPs and NPs are found in cell cytoplasm and nuclei Size dependent: significantly greater uptake of the smallest 25 nm NPs
				Membrane integrity	No significant effect
				Oxidative stress	No significant effect
				Cell viability	Significantly reduced
[24]	Co-culture (Caco-2/HT29-MTX)	PS	20 nm, 200 nm, and 2000 nm	Membrane integrity	Slightly impaired at high concentration (100 and 1000 μg/mL)
	Caco-2			Cell Viability	Size dependent: 20 nm NPs reduced cell viability while 200 nm and 2000 nm did not induce significant effect
[25]	Caco-2 monoculture, mucus co-culture (Caco-2 cell/HT29-MTX) & M cell model (Caco-2 cell/Raji B/ gut-associated lymphoid tissue (GALT)	PS	1 μm, 4 μm, 10 μm	Uptake	Size dependent: 1 and 4 μm >10 μm Co-culture > monoculture
				Cell viability	Caco-2 monoculture Size-dependent: 1 μm > 4 and 10 μm

Reference	Biological models	Polymer	Size	Biological effect	Important Findings
[26]	Caco-2 & HePG2	PE, PP, PVC, PET	1 μm and 4 μm, 10 and 20 μm	Uptake	Size dependent: 1 and 4 μm > 10 and 20 μm
				Cell viability	Significant decreased by PVC particles at high concentration (> 75 mg/mL) PE was slightly toxic at high concentrations only in HepG2 cells PE (in Caco-2), PP and PET were non-toxic
[27]	HRT-18	PS	NR	Oxidative stress	No significant effect
				Cytotoxicity	Increased cytotoxicity
				Inflammatory response	Increased expression of IL-8
				Gene expression	Up-regulation of *IL-8* gene No significant effect on *IL-18, IL-1β, MAPK1/ERK* and *HPRT* genes
[28]	HIEC-6 & CCD-841CoN	PS	0.1 μm, 0.5 μm, 1 μm, and 5 μm	Uptake	Uptake: HIEC-6 > CCD841CoN Size dependent: 0.1, 0.5, and 1 μm > 5 μm
				MPP	Decreased MPP
				Oxidative stress	HIEC-6 Size dependent: 0.1 μm > 0.5, 1, and 5 μm particles CCD841CoN Significant decreased ROS by 0.1, 0.5 and 5 μm
				Cell viability	No significant effect
				Apoptosis	HIEC-6 > CCD841CoN
[29]	RKO, HT-29, HCT-116, HIEC-6	PS	100 nm	Uptake	NPs are found in lysosomes
				Apoptosis	Induced early apoptosis
[30]	Co-culture (Caco-2/HT29-MTX-E12)	PS	50 nm and 0.5 μm	Uptake	No transport across barrier observed
				Membrane integrity	No significant effect
				DNA damage	No significant effect
				Cell viability	No significant effect

Reference	Biological models	Polymer	Size	Biological effect	Important Findings
[31]	Intestinal organoids	PS	1 μm, 500 nm, 100 nm, and 30 nm	Uptake	Endocytosis & transcytosis Size dependent: Smaller particle showed higher uptake rate
				Membrane integrity	SDM-induced monolayer without M cells (enterocytes, goblet cells, EECs, and Paneth cells) Size dependent: impairment induced by 30 nm NPs MDM-induced monolayers with M cells (enterocytes, goblet cells, EECs, and Paneth cells, M cells) Size dependent: impairment induced by 30 and 100 nm NPs
				Inflammatory response	Size and concentration dependent: increased expression of cytokines (TNFα, IL-6 and IL-8) at high concentration (>500 μg/mL) in the 100 nm and 30 nm NPs
[5]	Intestinal organoids from human induced pluripotent stem cells (hiPSCs)	PS	~50 nm	Uptake	Endocytosis
				Oxidative stress	Significantly increased
				Cytotoxicity	Mild cytotoxicity at high concentration (200 μg/mL)
				Inflammatory response	Significantly increased expression of NF-κBp65, IL-8, α-defensin-5
				Apoptosis	Significantly increased
[35]	HT-29	PS	3 μm and 10 μm	Oxidative stress	Time dependent: Induced oxidative stress at acute exposure (24 hours) and started to reduce in prolonged sub-chronic exposure up to 48 days
				DNA damage	Time dependent: induced DNA damage in acute exposure and became not evident in sub-chronic exposure after up to 28 days of exposure
				Cell viability	Time dependent: significant decrease in sub-chronic exposure

Reference	Biological models	Polymer	Size	Biological effect	Important Findings
[33]	CCD-18Co	PS	0.5 μm, and 2 μm	Uptake	Endocytosis Size dependent: 0.5 μm > 2 μm
				Mitochondrial activity	No significant effect
				Oxidative stress	Size dependent: 0.5 μm > 2 μm Significant induction of oxidative stress by NPs up to 4 weeks of exposure time and started to decrease
				Cytotoxicity	No significant effect
[34]	SNU-1	PS	50 nm, 100 nm, 200 nm, 500 nm, 1000 nm and 5000 nm	Uptake	NPs are found in cytoplasm and cell membrane Size dependent: smaller particle showed higher uptake rate
				Cell viability	Size dependent: 50 nm particles were generally more toxic to cells than larger particles
				Apoptosis	Size dependent: 50 > 5000 > 1000 > 500 > 200 > 100 nm

NR, Not reported; MPs, microplastics; NPs, nanoplsatics; MPP, Mitochondrial membrane potential; PVC, Polyvinyl Chloride; PC, Polycarbonate; PE, Polyethylene; PS, Polystyrene; PET, Polyethylene Terephthalate; PP, Polypropylene.