


A Comparative Systematic Analysis of The Influence of Microplastics on Colon Cells, Mouse and Colon Organoids

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Abstract

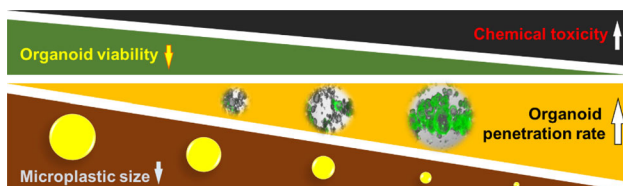
BACKGROUND: Microplastics (MPs) are small fragments from any type of plastic formed from various sources, including plastic waste and microfibers from clothing. MPs degrades slowly, resulting in a high probability of human inhalation, ingestion and accumulation in bodies and tissues. As its impact on humans is a prolonged event, the evaluation of its toxicity and influence on human health are critical. In particular, MPs can enter the human digestive system through food and beverage consumption, and its effect on the human colon needs to be carefully examined.

METHODS: We monitored the influence of small MPs (50 and 100 nm) on human colon cells, human colon organoids and also examined their toxicity and changes in gene expression in vivo in a mouse model.

RESULTS: The data suggested that 5 mg/mL concentrations of 50 and 100 nm MPs induced a > 20% decrease in colon organoid viability and an increase in the expression of inflammatory-, apoptosis- and immunity-related genes. In addition, in vivo data suggested that 50 nm MPs accumulate in various mouse organs, including the colon, liver, pancreas and testicles after 7 d of exposure.

CONCLUSION: Taken together, our data suggest that smaller MPs can induce more toxic effects in the human colon and that human colon organoids have the potential to be used as a predictive tool for colon toxicity.

Graphical abstract



Keywords Microplastics · Colon organoids · Differentiation · Normal colon cells · Toxicity

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1 Introduction

The impact of microplastics (MPs) on human health has become a great concern as their abundance in the environment has increased dramatically [1, 2]. MPs can be defined as any type of plastic less than 5 mm in length. [3, 4]. MPs cause serious pollution in natural ecosystems and are derived from a variety of sources, including clothing, cosmetics, industrial processes and food

packaging. As most MPs decompose very slowly in undisrupted natural environments, their impact on the environment, ecosystem and human health needs to be evaluated carefully given their long-term existence in nature [5, 6].

Humans can be exposed to MPs in various ways, including through the skin, digestive system and airways[7–9]. In particular, small MPs from food packaging and other plastic products, including straws, can enter the human body through the digestive system, which can influence human organs, including the human colon[10–12]. Therefore, systematic evaluation of the effect of small MPs on human colon cells and human colon organoids is important to provide valuable information about the effects of MPs on human health.

Human organoids are simplified and miniaturized three-dimensional assemblies of cells that show realistic micro-anatomy of actual organs[13, 14]. Organoids can be derived from either stem cells or cells from human/animal tissues and can be maintained and differentiated for several days up to several years[15]. Toxicity studies with animals have become difficult due to ethical issues, and because organoids closely mimic actual human organ structures, they can serve as a valuable research tool for studying complex human organs, including the influences of various substances on human organs[16, 17].

Here, we evaluated the effect of MPs on human colon fibroblasts, human colon organoids and a mouse model. By comparing the size-dependent impact of MPs on fibroblasts, organoids and within an *in vivo* system, information on how accurately human fibroblasts and organoids can predict *in vivo* toxicity was deduced. In addition, the potential of using human organoids as a predictive toxicity system is assessed.

2 Materials and methods

2.1 MPs preparation and characterization

Fifty and 100 nm microplastic beads conjugated with fluorescent dyes (Fluoresbrite® dyed particles, MP) were purchased from Polysciences (Warrington, PA, USA). MPs were sterilized by autoclaving and stored at 4 °C before the experiment. Field emission scanning electron microscopy (FE-SEM) images of MPs were obtained with a Sigma HD camera (Carl Zeiss AG, Jena, Germany). The particle size and zeta potential of MPs were obtained with a Litesizer 500 particle size analyzer (Anton Paar, Graz, Austria).

2.2 MPs cytotoxicity assay using CCD18-co cells

Normal human colon fibroblast CCD18-Co cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were maintained with Eagle's minimum essential medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific) and 1% penicillin–streptomycin (Thermo Fisher Scientific) in a 37 °C, 5% CO₂ incubator (Thermo Fisher Scientific). For the MPs-induced cytotoxicity test, CCD-18Co cells were seeded in 96-well cell culture plates at a density of 5000 cells/well (Corning, Corning, NY, USA). After 24 h of seeding, the cells were treated with the designated concentrations of 50 and 100 nm MPs (0.008, 0.04, 0.2, 1, 5 and 10 mg/mL). After 48 h of incubation, cell viability was monitored with a cell counting kit (CCK-8, Dojindo, Kumamoto, Japan). For the cell proliferation inhibition assay, 5,000 CCD-18Co cells were seeded on 96-well plates, and 5 mg/mL 50 and 100 nm MPs were added. After 12, 24, 72, 120 and 168 h, cell viability was monitored with CCK-8. For dead cell staining, 50 and 100 nm MPs-treated CCD-18Co cells were incubated for 48 h, and 4 μM ethidium homodimer-1 (EthD-1, Thermo Fisher Scientific) was added to the cells. Fluorescence images of cells were obtained with a Lionheart FX automated microscope (BioTek, Winooski, VT, USA).

2.3 Human colon organoid culture

Human colon organoids were suspended in an ice-cold mixture of 40% human colon organoid growth media (Stemcell Technologies, Vancouver, Canada) and 60% Matrigel (Corning). Colon organoid crypts were plated in a 96-well plate and incubated at 37 °C for 10 min. Next, 100 μL human colon organoid growth media was added and cultured at 37 °C; the medium was changed every other day. Organoids were passaged every 7–10 d with gentle cell dissociation reagents (Stemcell Technologies). Human colon organoids on 96-well cell culture plates were treated with the designated concentrations of MPs.

2.4 Human colon organoid staining and viability assay

For live/dead staining, colon organoids were incubated with 20 mM calcein-AM (green, Thermo Fisher Scientific) and 10 mg/ml EthD-1 (red, Thermo Fisher Scientific) for 60 min at 37 °C and then imaged by microscopy (Eclipse Ti2, Nikon, Tokyo, Japan). Additionally, 50 nm and 100 nm MPs were GFR-tagged colon organoids, and CCD-18Co cells only confirmed dead cells by EthD-1. For the viability assay, the colon organoids and CCD-18Co cells

were quantitatively analyzed using the CellTiter-Glo® 3D cell viability assay kit (Promega, Madison, WI, USA). At the indicated concentration points after MPs treatment, the luminescence generated by the reaction was measured using a microplate reader (SpectraMax iD5, Molecular Devices, San Jose, CA, USA) and presented as a dose–response curve. The IC₅₀ values of the MPs were determined from concentration-dependent inhibition curves using GraphPad Prism software (GraphPad Software, La Jolla, CA, USA).

2.5 Immunofluorescence (IF) staining

For IF staining, organoids were fixed in 4% paraformaldehyde for 30 min at room temperature, permeabilized, blocked with blocking solution (1% serum + 0.1% Triton X-100 in Dulbecco's phosphate buffered saline), and then stained with the primary mucin-2 antibody (Thermo Fisher Scientific) at 4 °C for 16 h and then the RFP-conjugated secondary antibody (Thermo Fisher Scientific) at room temperature for 2 h. Nuclei were counterstained with 4,6-diamidino-2-phenylindole dihydrochloride (Invitrogen, Waltham, MA, USA), and images were acquired on a microscope.

2.6 Total RNA isolation and quantitative PCR

Total RNA isolation of colon organoids was performed using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocols. RNA quality and quantity were assessed using a NanoDrop 2000 instrument (Thermo Fisher Scientific). RNA (2 µg) was reverse transcribed to cDNA using the RT2 First Strand Kit (Qiagen) according to the manufacturer's protocols. The expression of 84 key colon inflammation-related genes was evaluated using Human CD RT² Profiler PCR Arrays (#PAHS-169Z; Qiagen, Table S1) and SYBR Green chemistry (RT2 SYBR Green ROX qPCR Mastermix; Qiagen) according to the manufacturer's protocols using the Roter gene Q instrument (Qiagen). The expression level of each gene was normalized to the geometric mean of five housekeeping reference genes (ACTB, B2 M, GAPDH, HPRT1 and RPLP0) based on RefFinder algorithm (GeneGlobe, Qiagen) analysis.

2.7 Animal care and experimentation

All animal experiments were carried out using C57BL/6 mice according to the established guidelines of the Institutional Animal Care and Use committee of the Korea Research Institute of Chemical Technology. Seven-week-old C57BL/6 male mice were purchased from Orient Bio. (Seongnam, Korea). The mice were placed in a specific

pathogen-free animal room under controlled conditions (temperature, 23 ± 2 °C and humidity, 55–60%) and a 12/12 h light/dark cycle. All animals were allowed to acclimate for at least 1 week before use. Standard laboratory animal feed was purchased from Biopia (Gunpo, Korea), and animal feed and water were freely provided. The 50 nm MPs dose was determined based on the concentration at which the highest toxicity was found in colon organoids. Mice were orally administered once on Day 0. The mice were exposed to MPs for a total of 7 d and were weighed before the intervention and on the final day of exposure. At the end of the exposure on the final day, the mice were euthanized, and the blood, colon, kidney and testes of the mice were collected. The plasma concentrations of glucose, aspartate transaminase (AST), alanine transferase (ALT), cholesterol (CHO), HDL-cholesterol, and LDL-cholesterol were measured by colorimetric assays using an automatic biochemical analyzer (Selectra 2, Vital Scientific N.V., The Netherlands). The plasma concentrations of interleukin 8 (IL-8) were measured by a colorimetric assay using an IL-8 ELISA kit (R&D Systems, Minneapolis, MN, USA) with concentrations expressed in units of ng/mL. All in vivo experimental procedures were approved by the Animal Research Committee of the Korea Research Institute of Chemical Technology (approval number: 2022-7A-09–01).

2.8 Tissue histology

Paraffin Sects. (8 µm) of mouse colons were attached to poly-L-lysine-coated glass slides. After incubation at 60 °C for 30 min, the slides were dewaxed and hydrated stepwise using xylene followed by several solutions of distilled water containing decreasing amounts of ethanol (100–60%, vol/vol). The sections were stained with hematoxylin and eosin (H&E, Abcam, Cambridge, UK) and an Alcian blue periodic acid-Schiff (PAS) staining kit (Abcam) according to the manufacturer's protocol. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining was conducted according to the manufacturer's protocol (Abcam).

2.9 Statistical analysis

All data are expressed as the mean ± standard error, and the results were generated from three independent experiments (n = 4 ~ 8/group) with a minimum of three technical replicates unless otherwise stated. The statistical analysis included a one-way analysis of variance and t tests, which were performed using the GraphPad Prism program, Qiagen GeneGlobe and Excel. Values below $p < 0.05$ are considered statistically significant (i.e., * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

3 Results

3.1 MPs Characterization after autoclaving

MPs (50 nm and 100 nm) were autoclaved to remove potential contaminating factors before cell and organoid treatment. The size and surface charge of the MPs were monitored before and after autoclaving. The data suggest that autoclaving does not induce significant changes in MPs size (66.1 vs. 65.5 nm for 50 nm MPs and 113.7 vs. 111.8 nm for 100 nm MPs before and after autoclaving, respectively) or surface charge (-54.5 vs. -56.4 mV for 50 nm MPs and -55.6 vs. -56.2 mV for 100 nm MPs before and after autoclaving, respectively, Fig. 1A–C and Figure S1). The fluorescence intensity from fluorophores conjugated to MPs also showed no significant changes before and after autoclaving (Fig. 1D). Overall, these data indicate that autoclaving does not induce significant changes in MPs properties.

3.2 Effect of MPs on cytotoxicity in human normal colon cells

After MPs characterization, we monitored MPs-induced toxicity in normal colon CCD-18Co cells. Fluorescence from the MPs-conjugated fluorophore was detected in the cytosol and nucleus of CCD-18Co cells, suggesting that MPs can successfully penetrate/endocytosis into cells (Fig. 2A). TEM images of CCD-18Co cells incubated with 50 nm MPs also suggested MPs infiltration inside the cells (Fig. 2B). Forty-eight hours of exposure to 10 mg/mL 50 nm MPs induced a 13.2% decrease in CCD-18Co cell viability, whereas the same concentration of 100 nm MPs induced a 7.2% decrease in colon cell viability (Fig. 2C). Figure 2D shows that 168 h of exposure to 10 mg/mL MPs decreased CCD-18Co cell proliferation by 16.5 and 13.8%, respectively. Dead cell staining with EthD-1 also suggested only a small increase in the percentage of dead cells in the presence of 5 mg/mL 50 and 100 nm MPs compared with that of the control (Fig. 2E). Thus, our data indicate that although both MPs can successfully infiltrate cells, CCD-

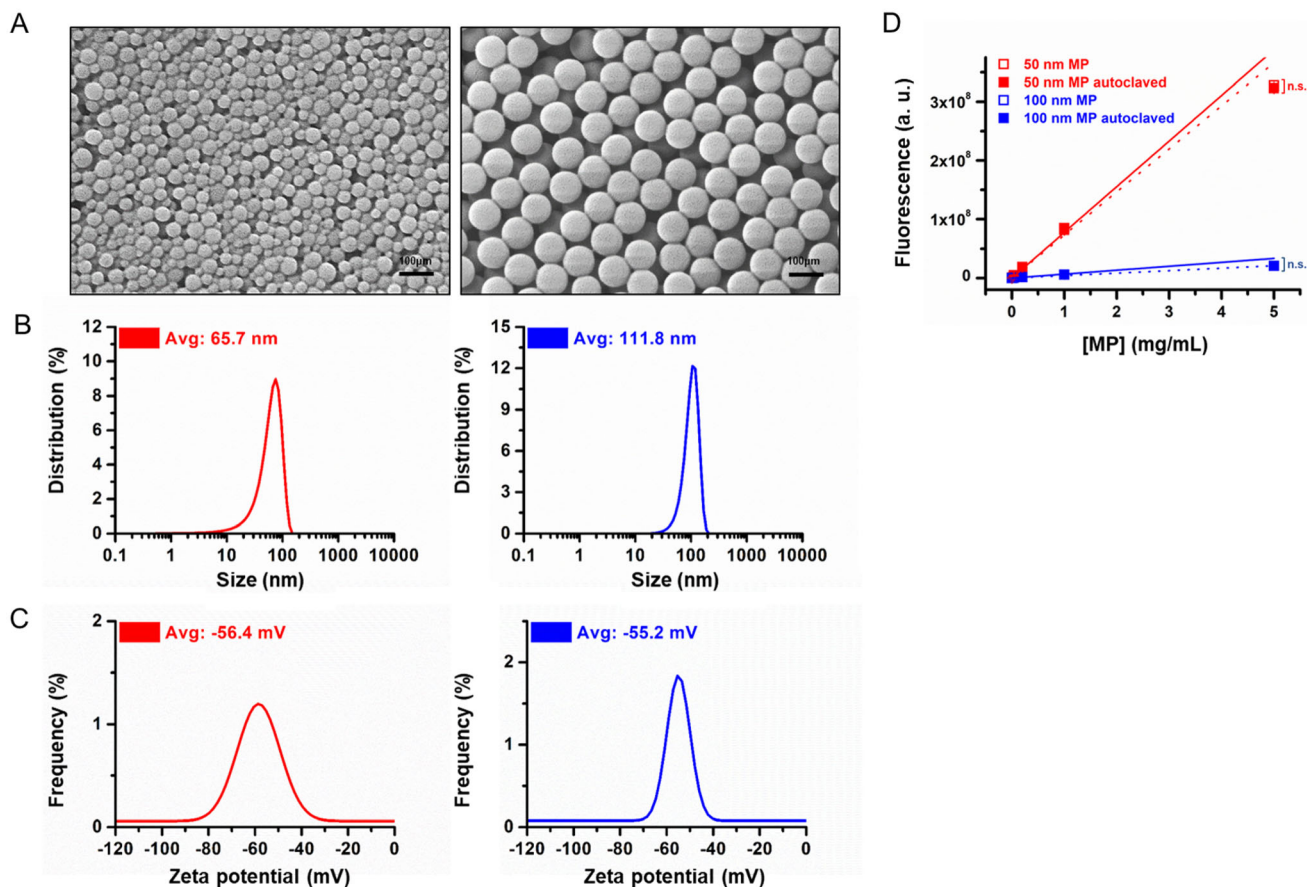


Fig. 1 Characterization of 50 and 100 nm MPs after autoclaving. **A** FE-SEM images of 50 (left) and 100 (right) nm MPs. **B** Size distribution of 50 and 100 nm MPs. **C** Zeta potential distribution of

50 and 100 nm MPs. **D** Fluorescence intensities from 50 and 100 nm MPs with increasing concentration. The results are presented as the mean \pm S.D. ($n = 6$)

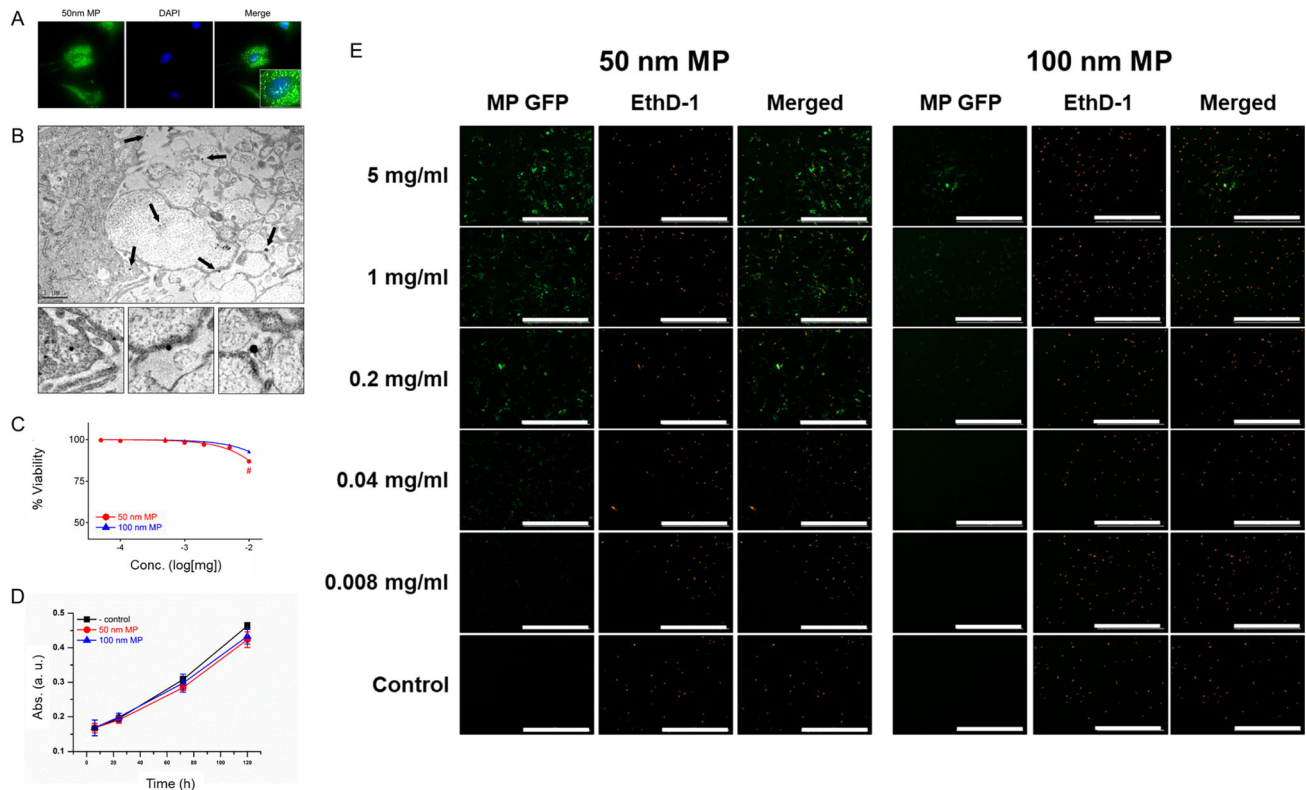


Fig. 2 Effect of MPs on human colon fibroblasts. **A** Fluorescence microscopic images of CCD-18Co cells after incubation with 50 nm MPs. **B** TEM images. **C** Viability. **D** Proliferation and **E**. Live-dead

18Co cell viability and proliferation do not change significantly after short (12 h) or long (168 h) exposure to MPs.

3.3 Effect of MPs on cytotoxicity in human colon organoids

Next, we monitored MPs-induced toxicity in human iPSC-derived colon organoids. Although fluorescence from both 50 and 100 nm MPs-conjugated fluorophores was detected inside colon organoids, more infiltration of 50 nm MPs inside colon organoids was observed compared with that of 100 nm MPs upon exposure to various concentrations (0.0003–5 mg/mL, Fig. 3A). We also confirmed that 50 nm MPs can infiltrate the cytosol of colon organoids, but MPs penetration into the nucleus of colon organoids was not observed with TEM (Fig. 3B). The effect of MPs exposure on gut-specific marker mucin-2 expression in colon organoids was monitored with immunofluorescence staining. Colon organoids treated with 50 and 100 nm MPs showed an increase in mucin-2 expression compared with that of the control (Fig. 3C). The effect of MPs on the viability of colon organoids showed a more prominent decrease in colon organoid viability when treated with 50 nm MPs compared with that of 100 nm MPs (Fig. 3D). The dead cell staining results suggested that 50 nm MPs

cell staining results of CCD-18Co cells after incubation with MPs. The scale bar represents 50 μ m. The results are presented as the mean \pm S.D. (n = 6)

induced a more prominent increase in the percentage of dead cells in colon organoids than that of 100 nm MPs (Fig. 3E). When comparing the size of colon organoids after exposure to MPs, 50 nm MPs induced a more prominent decrease in colon organoid size compared with that of 100 nm MPs (Fig. 3F). Taken together, 50 nm MPs induced more significant effects on human colon organoid viability, size and gut-specific marker expression than those of 100 nm MPs.

3.4 Effect of MPs on the expression of apoptosis-, immune response- and inflammation-related genes and cytokines in human colon organoids

As MPs-induced toxicity in human colon organoids was observed, we evaluated changes in gene and cytokine expression after incubation with MPs. The expression levels of 77 genes associated with inflammation, apoptosis, immunity, metabolism, the extracellular matrix (ECM), adhesion molecules and digestive secreted proteins were evaluated. The data suggested that out of 29 inflammation-related genes, the expression levels of 18 genes were increased more than twofold when incubated with 50 nm MPs (14 genes increased more than twofold with 100 nm MPs) compared with levels of control organoids (Fig. 4A).

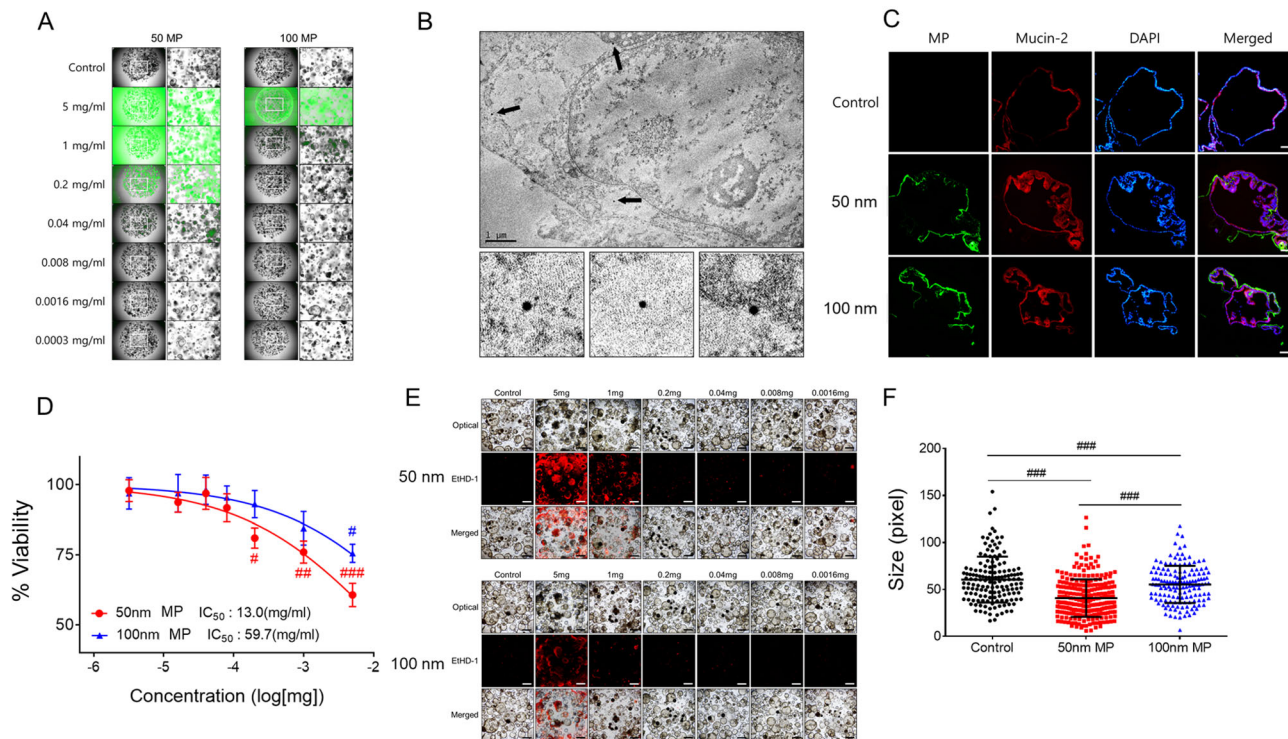


Fig. 3 Effect of MPs on viability of human colon organoids. **A** Fluorescence microscopic images. **B** TEM. **C** Fluorescence from MPs and mucin-2 in colon organoids. **D** % viability changes (with IC_{50} 13.0 mg/ml and 59.7 mg/ml for 50 nm and 100 nm MPs, respectively), **E** dead cells from EtHD-1 staining and **F** size changes

of colon organoids after incubation with 50 and 100 nm MPs. Fluorescence images were taken under magnification $\times 100$. The results are presented as the mean \pm S.D. (n = 6). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. control

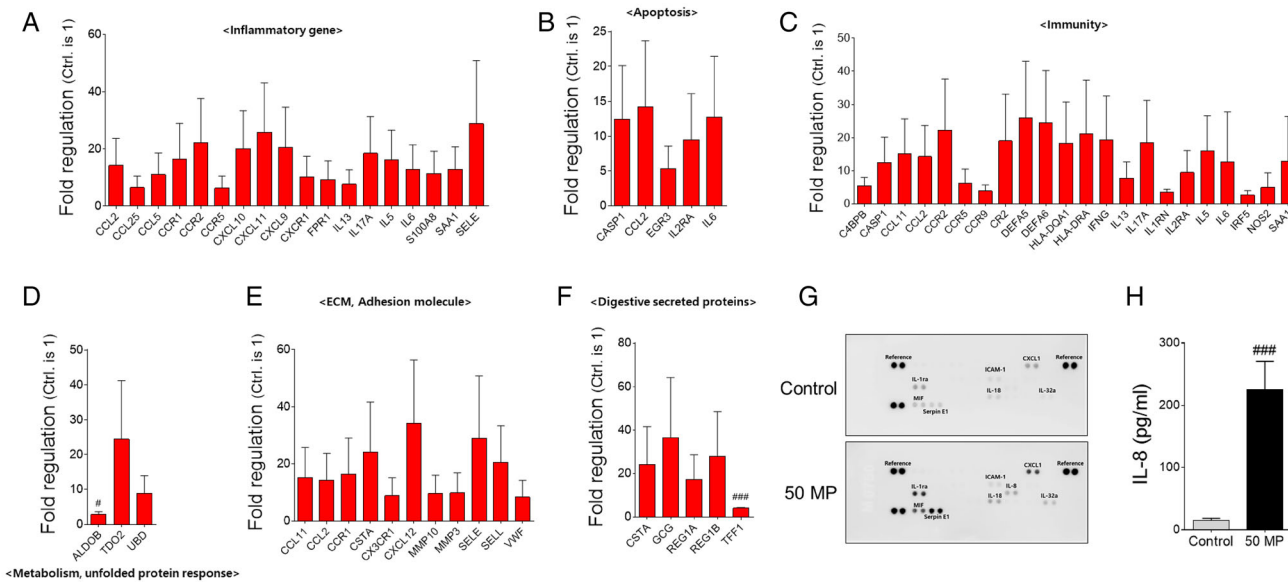


Fig. 4 PCR array results of colon organoids after incubation with 50 nm MPs. Changes in the gene expression of **A** inflammatory, **B** apoptosis, **C** immunity, **D** metabolism (unfolded protein response), **E** ECM and adhesion molecules, and **F** digestive secreted proteins of colon organoids after incubation with 50 nm MPs. **G** Dot blot results

of control and 50 nm MPs-treated organoids of key inflammatory-related genes. **H** Amount of IL-8 detected in control and 50 nm MPs-treated colon organoids. The results are presented as the mean \pm S.D. (n = 6). $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ vs. control

For apoptosis-related genes, the expression levels of 5 out of 10 genes were increased more than twofold with 50 nm MPs (4 genes showed increased expression with 100 nm MPs) compared with levels in the control (Fig. 4B). The 50 nm MPs-induced increase in 22 immune-related genes (out of a total of 32 genes) was increased more than twofold, whereas 100 nm MPs induced a greater than twofold increase in 17 genes (Fig. 4C). For genes related to metabolism and unfolded protein responses, both 50 and 100 nm MPs induced a greater than twofold increase in 3 genes out of 4 (Fig. 4D). The expression levels of 11 ECM and adhesion molecules were increased more than twofold when incubated with 50 nm MPs (7 molecules for 100 nm MPs) out of a total of 17 genes (Fig. 4E). MPs also induced an increase in digestive secreted proteins (> twofold for 5 genes with 50 nm MPs and 4 genes with 100 nm MPs out of a total of 6 genes, Fig. 4F). We also monitored changes in cytokine expression in colon organoids after incubation with MPs. Upon the addition of 50 nm MPs, the expression levels of interleukin-1 receptor antagonist (IL1-R α), C-X-C motif chemokine ligand 1 (CXCL1), macrophage migration inhibitory factor (MIF) and plasminogen activator inhibitor-1 (serpin E1) were increased in colon organoids compared with levels in the control (Fig. 4G). Noticeably, 50 nm MPs induced the expression of IL-8, which was not detected in control organoids (Fig. 4G). Exposure to 50 nm MPs also induced an increase in IL-8 in colon organoid culture media compared with that of the control based on an enzyme-linked immunosorbent assay (ELISA, Fig. 4H). However, fewer changes in gene expression related to inflammation, apoptosis, immunity and digestive secreted proteins were observed on colon organoids exposed to 100 nm (Supplementary Fig. S2). Taken together, our data suggest that 50 nm MPs induced increases in various apoptosis-, immune response- and inflammation-related genes in human colon organoids.

3.5 Effect of MPs on in vivo toxicity

We exposed mice to 50 nm MPs and monitored the accumulation of fluorophore-tagged MPs in organs. After 7 d of 50 nm MPs exposure, the amount of 50 nm MPs detected in mouse plasma increased dramatically compared with that after 2 h of exposure (Fig. 5A) compared to non-treated control (Supplementary Fig. S3). The amount of MPs accumulation was also evaluated after homogenization of each organ. The data also suggested that significant amounts of MPs penetrated into various organs, including the colon, liver, spleen and testes (Fig. 5A). H&E staining of various mouse organs after MPs exposure indicated that intense fluorescence signals from fluorophore-tagged MPs were detected in various organs (Fig. 5B). To evaluate the effect of MPs exposure on colon walls, alcian blue PAS

and TUNEL staining were conducted. The degree of PAS staining increased with MPs exposure, whereas no significant changes in alcian blue staining were detected (Fig. 5C). The TUNEL staining results suggested that cells in the colon wall were undergoing apoptosis after MPs exposure (Fig. 5D). Biochemical analysis of mouse blood after 7 d of 50-nm MPs exposure suggested that mice treated with MPs exhibited increased levels of plasma ALT and AST (Fig. 5E), but there were few or no changes in protein levels related to cholesterol and lipogenesis (glucose [GLU], cholesterol [CHO], high-density lipoprotein C [HDL-C] and low-density lipoprotein C [LDL-C]) compared with those of the control. Interestingly, similar to that of colon organoids, the IL-8 level in plasma was increased dramatically in mice after MPs exposure (Fig. 5F). However, no noticeable changes in mouse body weight, colon, thymus, testes or spleen weight were observed in mice treated with 50 nm MPs (Supplementary Fig. S4). Overall, our in vivo MPs toxicity results suggest that mice exposed to MPs show possible toxic effects on various organs.

4 Discussion

Previous studies have suggest that ingestion of MPs can induce gastrointestinal (GI) disease. Ingested MPs induces change in human gut microbiota as well as induce immunotoxicity [18–20]. MPs accumulation in human GI tract can lead to disruption of GI tract mucus and epithelium [20]. Therefore, effect of MPs consumption on human colon system is need to be carefully evaluated.

By systematically assessing the influence of 50- and 100-nm MPs on 2D cell culture and mouse and colon organoids, our data suggest that MPs size affects its influence. A previous study also suggested that the size of MPs is important in fish toxicity and larger MPs pose relatively little risk [21]; our data confirm that smaller MPs induce more severe toxic effects. Increased level of inflammatory, apoptosis and immunity related-genes in human colon organoids upon addition of MPs suggests that ingestion of MP may potentially induce cytotoxic effects in human GI tract. Ingested MPs leading to an upregulation of immune response genes causing inflammation and toxicity in human GI tract. MPs also induces direct increase of apoptotic related genes leading to a cell death in GI system. Although exact mechanism of MP induced toxicity is still need to be examined, it has been reported that MPs induces inhibition of DNA replication, cell cycle arrest in S phase and G2/ M phase during cell replication inducing cytotoxicity in zebrafish models [22]. High concentration of MPs also induces rupture of lipid bilayer of cell plasma membrane as well as inducing programmed cell death [23].

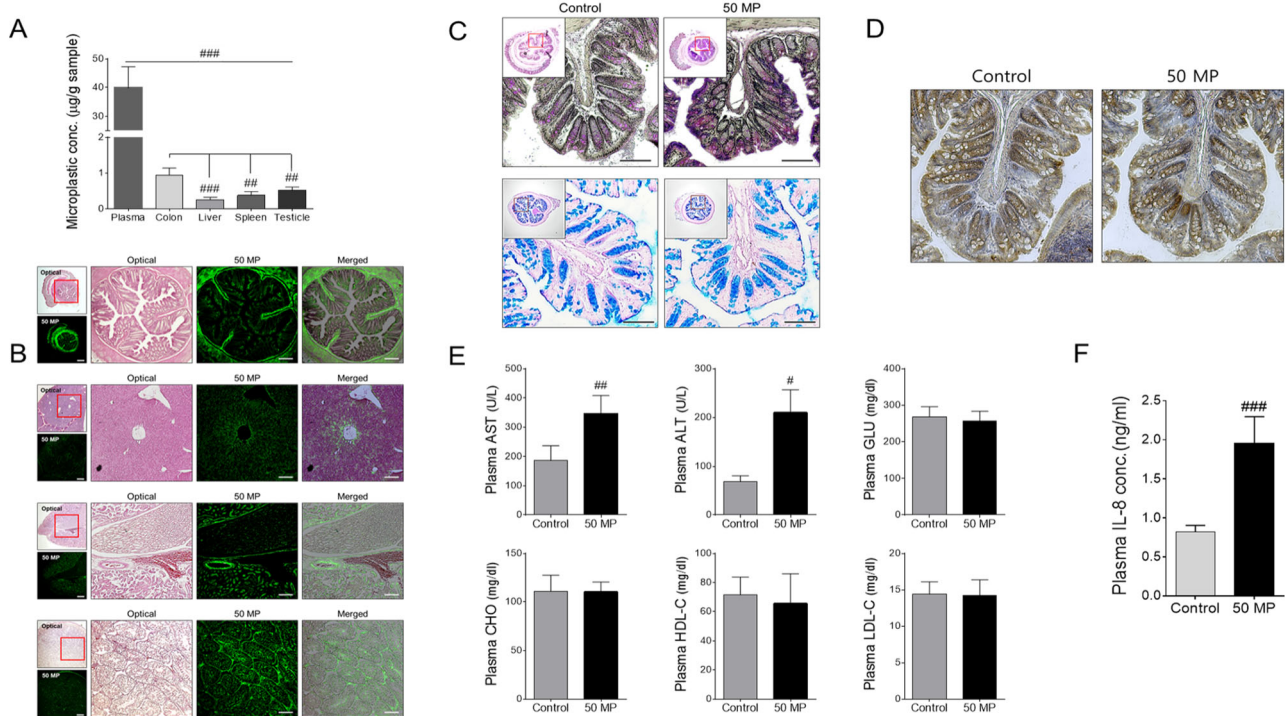


Fig. 5 Effect of 50 nm MPs on toxicity in C57BL/6 male mice. **A** Concentration of MPs detected in mouse plasma, colon, liver, spleen and testicles. **B** Fluorescence of MPs detected in mouse tissue. **C** PAS and alcian blue staining and **D** TUNEL staining of 50 nm MPs treated mouse colon tissue. **E** Plasma AST, ALT, GLU, CHO, HDL-

C, and LDL-C concentrations. **F** Plasma IL-8 concentrations after incubation with 50 nm MPs. The scale bar represents 200 µm. The results are presented as the mean ± S.D. (n = 6). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. control

Exposure to MPs also causes inflammation, oxidative stress and changes in lipid metabolism [24].

Traditionally, 2D human cell culture has been used to evaluate the toxicity of various materials to predict the effect on human health [25]. This type of cell culture has various advantages such as the ease of growing cells and the ability to obtain a reliable result that is relatively less dependent on various experimental conditions. However, translating 2D in vitro results to impacts on humans is a serious obstacle and often leads to false predictions. [26, 27] In vivo experiments are an essential and terminal experimental tool in advance of human clinical trials to evaluate the toxicity of various environmental hazards [28, 29]. Unlike 2D cell culture, in vivo experiments include human-like processes such as blood flow, adsorption, digestion, metabolism and excretion (ADME) mechanisms. This allows a more accurate prediction of the effect of various materials on human health. However, there is a growing concern regarding the use of countless animals in biological experiments [30, 31]. In addition, there is still a biological complexity gap between human and experimental animals; therefore, in vivo experiments also have limitations [32, 33]. Recently, there has been growing interest and ongoing research on using human organoids to evaluate the effects of various materials on human health

[34, 35]. Human stem cell-derived organoids can be differentiated into various organs mimicking complex and precise structures. Several recent studies have shown that human stem cell-derived organoids can be used as valuable and accurate tools for evaluating the impact of drugs, toxins and various materials on human health [36, 37]. Recent advances in organoid technology enable high organoid-human correlations and show potential for replacing in vivo experiments. Organoids still have limitations in that although they are derived from human stem cells and closely mimic human organs, they lack the blood flow and ADME of live organisms. [38] However, there is a growing effort to introduce blood vessels and fluid flow into human organoids, and more complex and human-mimicking organoids are expected to be introduced [39].

Our data also suggest a promising future for using organoids as human health impact prediction tools. Compared with the 2D cell culture system, the experimental results of the MPs effect on colon organoids more closely mimicked the in vivo experimental data. Concentration-dependent viability changes in response to MPs were more dramatically observed in human colon organoids than in 2D cultures. In addition, an increase in mucin-2 expression levels in human colon organoids as well as an increase in toxicity upon exposure to MPs were also observed in the

in vivo experiment. Although it is too early to conclude that human colon organoids can replace in vivo experiments due to a lack of clinical data on the MPs effect on the human colorectal system, our data suggest that advances in colon organoid culture may help to reduce the use of in vivo experiments for predicting human health impacts. In addition, human-origin colon organoids can overcome in vivo experimental vs. human clinical discrepancies arising from species differences.

In summary, we systematically evaluated the effect of MPs on human colon cells, human colon organoids and an in vivo mouse models. Our data suggest that smaller MPs induce more toxic effects and that the results in human colon organoids are more similar to those in the in vivo model than those in human colon cells. Overall, human colon organoids can be used as valuable predictive tools for assess MPs-induced toxicity in humans.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13770-022-00496-8>.

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Declarations

Conflict of interest The authors have no potential conflicts of interest.

Ethical statements All animal experiments were carried out using CD-1(ICR) mice according to the established guidelines of the Institutional Animal Care and Use committee of the Korea Research Institute of Chemical Technology. All animals were maintained under a room illuminated daily from 07:00 to 19:00 (12:12 h light/dark cycle), with a temperature of 23 ± 1 °C, a ventilation of 10–12 times per hour, and a humidity of $55 \pm 5\%$. All in vivo experimental procedures were approved by the Animal Research Committee of the Korea Research Institute of Chemical Technology (approval number: 2022-7A-09-01).

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