

# The Cytotoxicity of Micro-plastics on Co-culture Models of Intestinal Cells and Investigation of Oxidative Stress Mechanisms

Xuan Wu

RCF Experimental School, Beijing 100000, China.

**How to cite this paper:** Xuan Wu. (2025)

The Cytotoxicity of Micro-plastics on Co-culture Models of Intestinal Cells and Investigation of Oxidative Stress Mechanisms. *Health and Prevention Journal*, 2(1), 92-102.

DOI: 10.26855/hpj.2025.12.008

**Received:** October 2, 2025

**Accepted:** November 23, 2025

**Published:** December 22, 2025

\***Corresponding author:** Xuan Wu. RCF Experimental School, Beijing 100000, China.

## Abstract

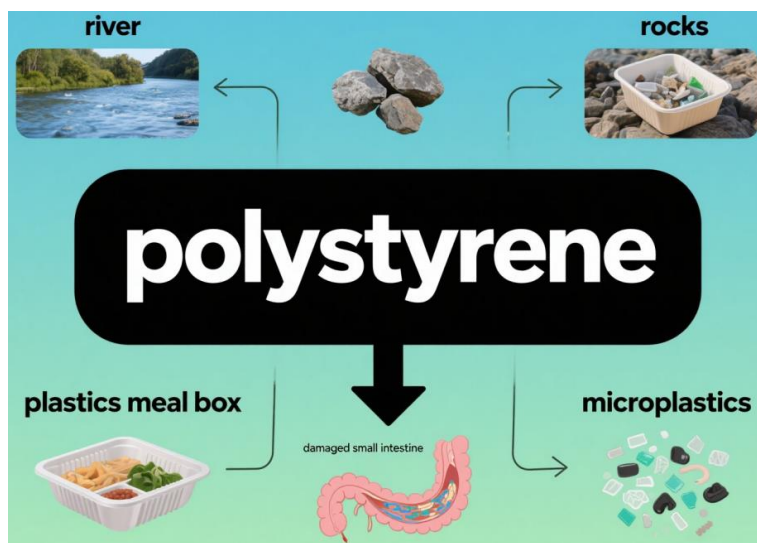
Microplastics (particles < 5  $\mu\text{m}$  in diameter) are pervasive environmental contaminants resulting from human activities. Common sources include polystyrene (PS) in disposable food containers, polyvinyl chloride in medical masks, and polyethylene terephthalate (PET) in synthetic textiles. Human exposure to microplastics frequently occurs through ingestion and inhalation, often without conscious awareness. This study investigated the effects of different sizes (80 nm and 200 nm) and concentrations of PS microplastics on the cell cycle and apoptosis of HCT116 colorectal carcinoma cells. Additionally, the potential of chitosan to mitigate PS-induced cytotoxicity was evaluated in both intracellular and extracellular contexts. Assessments were conducted using MTT assays, cell cycle analysis, and apoptosis assays. MTT results indicated that higher concentrations of 80 nm PS microplastics significantly reduced HCT116 viability, whereas 200 nm particles had comparatively lesser effects. Cell cycle analysis revealed that exposure to 1  $\mu\text{g}/\text{mL}$  PS significantly increased the proportion of cells in the G1 phase and decreased those in the S phase. These concentrations correspond to typical levels released from disposable plastic food containers when used with hot food (60–90°C). Apoptosis assays via flow cytometry confirmed that 80 nm PS microplastics induced more severe cytotoxic effects than their 200 nm counterparts. Notably, the addition of chitosan markedly reduced the toxicity of both PS variants. These findings suggest that chitosan may serve as a protective agent against microplastic-induced intestinal cell damage, offering a potential basis for future therapeutic strategies to safeguard human intestinal health.

## Keywords

Microplastics; intestines; cytotoxicity; HCT116

## 1. Introduction

Microplastics (MPs), defined as plastic particles under 5  $\mu\text{m}$ , and nanoplastics (1–100 nm), are persistent environmental pollutants due to their non-biodegradability. They are ubiquitous in rivers, soil sediments, and everyday consumer products (Figure 1). Recent studies using pyrolysis-gas chromatography have detected plastic particles in human blood at concentrations up to 1.6  $\mu\text{g}/\text{mL}$  [2]. Oral exposure to MPs has been linked to organ dysfunction in animal models, evidenced by altered aminotransferase (AST) levels and reduced total antioxidant capacity (T-AOC) [3]. Moreover, MPs can act as carriers for environmental pollutants such as Benzo[a]pyrene (BAP), leading to intestinal villi loss and inflammation [4].



**Figure 1. The correlation of PS microplastics with the surrounding environment.**

Polystyrene (PS) is a common microplastic found in transparent bottles, CDs, and food containers. Its inadvertent ingestion poses a significant threat to intestinal health. MPs enter cells primarily via endocytosis [5], where they disrupt organelle function—particularly of lysosomes—and promote lipid peroxidation. The rough surface of MPs and reactive oxygen species (ROS) generation can compromise lysosomal membrane integrity, releasing destructive enzymes into the cytoplasm. Subsequent iron release may trigger ferroptosis, further damaging cells [6]. ROS-induced mitochondrial DNA mutations can activate caspases, amplify oxidative stress and lead to apoptosis [7]. These mechanisms may contribute to symptoms such as fatigue, malnutrition, and intestinal spasms.

Despite these insights, the precise cytotoxic pathways and threshold concentrations of PS-induced apoptosis and cell cycle arrest remain unclear. This study aims to elucidate the effects of PS microplastics on intestinal cells and evaluate the protective role of chitosan.

## 2. Methods & Materials

### 2.1 Materials Preparation

Unibead Uniform Polystyrene Microplastics used in the experiments were purchased from Tianjin Bestill Chromatography Technology Development Center, China. Flow cytometer of Novocyte was used in the experiment. Microplate reader was purchased from SpectraMax i3X. Propidium Iodide (PI), Annexin V and binding buffer were bought from KeyGEN BioTECH. The polystyrene microspheres size was 0.2  $\mu\text{m}$  as well as 0.08  $\mu\text{m}$ . Before using the PS microplastics in the cellular toxicity experiments, 1000  $\mu\text{L}$  of each size of PS microplastics were extracted using a pipette into the centrifuge tubes. The PS microplastics in the tubes were sterilized. The colorectal carcinoma HCT116 was purchased from the American Type Culture Collection (ATCC.org). The reason to use colorectal carcinoma cells instead of normal intestinal cells was to reduce the subculture period, ensuring rapid passage of new cells to the next generation for subsequent experiments. HCT116 had been cultivated for 48 hours in the Petri dishes before the initiation of the actual experiment. 15 mg/mL (1.5% w/v) of chitosan was used in the cell apoptosis test. The chitosan was mixed with water evenly and a membrane filter was then used to extract the solution. The chitosan solution was strictly sterilized as well before being used in the cell apoptosis test.

### 2.2 Cell Culture

The experiments were conducted under a sterilized environment—Clean Bench. All the experimental containers were first sprinkled by ethanol and then processed by an alcohol lamp in the clean bench to ensure fully sterilized conditions. HCT116 were adherent cells, as a result, the superficial culture medium was poured and then the remaining residues were rinsed with Phosphate-Buffered Saline (PBS). 1 mL of trypsin was added to the Petri dish and the dish was placed into the cell culture incubator for 1 min at 37°C to allow HCT116 to shed off. 2 mL of complete medium

(1% of penicillin, streptomycin, amphotericin, 10% of FBS, 89% of RPMI1640) was then added into the Petri dish and followed by pipetting all the solution into the centrifuge tube. The extracted 3 mL solution in the centrifuge tube was then centrifuged at 1200 rpm for 3 minutes. After discarding all the supernatant, add 3 mL of complete culture medium into the centrifuge tube to make the cell suspension. 100  $\mu\text{L}$  of cell suspension was transferred into another 1.5 mL centrifuge tube by a pipette and followed by 400  $\mu\text{L}$  of complete culture medium adding into the tube to dilute the solution. The dilute solution was used in a hemocytometer to calculate the concentration of HCT116 in the original centrifuge tube. The results showed that the concentration was  $4.35 \times 10^3$  cells/ $\mu\text{L}$  in the original tube. 40 cells/ $\mu\text{L}$  of solution was obtained by diluting 100  $\mu\text{L}$  of  $4.35 \times 10^3$  cells/ $\mu\text{L}$  solution with 11 mL of complete culture medium. The solution of diluted HCT116 containing complete culture medium was adding into the 96-well plate and each well was added by 200  $\mu\text{L}$  of the dilute solution (8000 cells). 6  $\mu\text{L}$  of the diluted solution was equally distributed into 4 disks for cell subculture and used in the later experiments.

### 2.3 Cytotoxicity Test by MTT

In the experiment, MTT (mono-tetrazolium salt) was the method to testify cell activity, and the mechanism was based on the activity of mitochondria dehydrogenase to reflect the activity of HCT116. 1000  $\mu\text{L}$  of both sizes of microplastics including 200 nm and 80 nm had a concentration of 25000  $\mu\text{g}/\text{mL}$ . Experimental groups including 1  $\mu\text{g}/\text{mL}$ , 10  $\mu\text{g}/\text{mL}$  and 100  $\mu\text{g}/\text{mL}$  for each size of microplastics were obtained by dilution of microplastics solution in the centrifuge tubes with normal culture medium (no serum). The superficial fluids in each well of the 96-well plate were aspirated out and 200  $\mu\text{L}$  of each concentration of PS microplastics solution was added into the corresponding well (Figure 2). The two control groups of both sizes of the PS microplastics were added by 200  $\mu\text{L}$  of normal culture medium in each well as well. In one 96-well plate, both experimental groups (for each concentration and size of PS microplastics) and control groups contained 6 wells for each concentration of PS microplastics to minimize experimental errors and to avoid experimental randomness. After culturing the 96-well plates in the cell culture incubator for 24 hours, all the superficial fluids above the adherent cells were discarded. 100  $\mu\text{L}$  of MTT was added into each well of the plate and dyed the mitochondria of the cells for 4 hours without light. After waiting for 4 hours, the MTT solution was removed followed by 100  $\mu\text{L}$  of formazan added into each well. As a result, the extent of absorption of light by HCT116 in the micro-plate reader can then be obtained to identify the activity of HCT116 under various concentration of PS microplastics.

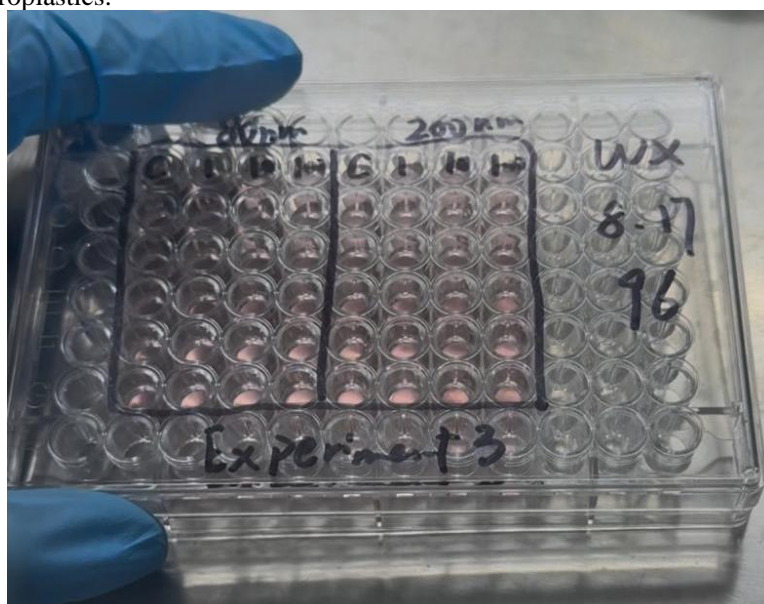


Figure 2. Image from the experiment which showed preparation of MTT test.

### 2.4 Cell Cycle Phases by Flow Cytometry

In the cell cycle test, RNase A was combined with propidium iodide (PI) to monitor different phases of mitosis. Propidium iodide was a fluorescent dye which could insert into the double helix DNA and sent signals to the

equipment of its concentration. Rnase A played a crucial role in breaking down RNA molecules in the cell into smaller nucleotides so that propidium iodide could only specifically target on DNA molecules to reflect cell cycle. Three 6-well plates were seeded by HCT116 to test the phases of the cell cycle. The process of seeding was almost the same as for the 96-well plates. Each plate of cells was rinsed by 200 mL of PBS and added 1 mL of trypsin, waiting for 1 minute. The following step was to add 2mL of complete culture medium and to centrifuge for 3 min at 1200 rpm. The superficial fluids were discarded, and 6 mL of complete culture medium was added into the 15 mL centrifuge tube with HCT116. 2 mL of complete culture medium range from to 500  $\mu$ L of HCT116 from the centrifuged tube were added into each well of the 6-well plates by ratio of 4:1. Finally, the plates were placed back into the incubator for a whole night. After 24 hours, all the superficial fluids of the complete culture medium were aspirated out from the 6-well plates. Two plates corresponded to 80 nm, and one plate corresponded to 200 nm sizes of PS microplastics. Each plate has 6 wells, and the upper three wells were control groups, and the downward wells were experimental groups with selective concentration of PS microplastics after MTT test in the 96-well plates.

The selection was based on the data given in the cell cytotoxicity test. 1  $\mu$ g/mL of 80 nm microplastics as well as 1  $\mu$ g/mL of 200 nm microplastics were used to test the cell cycle of HCT116 because they could both influence HCT116 activity but keep them alive to be tested. Each well was added by 2 mL of the corresponding solution of microplastics. The control group was added by 2 mL of culture medium. After cultivation for 24 hours, the 6-well plates were taken out with the superficial fluid removed. The HCT116 in each well was extracted using 500  $\mu$ L of trypsin and 1000  $\mu$ L of complete culture medium. The digestion of trypsin to strip off HCT116 from the wall. The mixture was then added into centrifuge tubes. After centrifugation at 2000 rpm for 5 minutes, the fluids in the centrifuge tubes were replaced by 70% ethanol (still 4-8°C liquid) to stabilize the shape and size of HCT116. The 4-8°C 70% ethanol was prepared by mixing 7 mL of ethanol with 3 mL of distilled water. The stabilization lasted for 2 hours and after that, the ethanol was discarded after centrifugation and 2 mL of PBS was added to rinse the HCT116. The centrifuge tubes were centrifuged at 1000 rpm for 3 minutes. RNase A and Propidium Iodide were mixed in a ratio of 1:9 to dye the HCT116. Each centrifuge tube was added by 500  $\mu$ L of PI/RNase A in a dark environment and waited for 50 minutes. Finally, each centrifuge tube was placed on a flow cytometer to test the complete cell cycle.

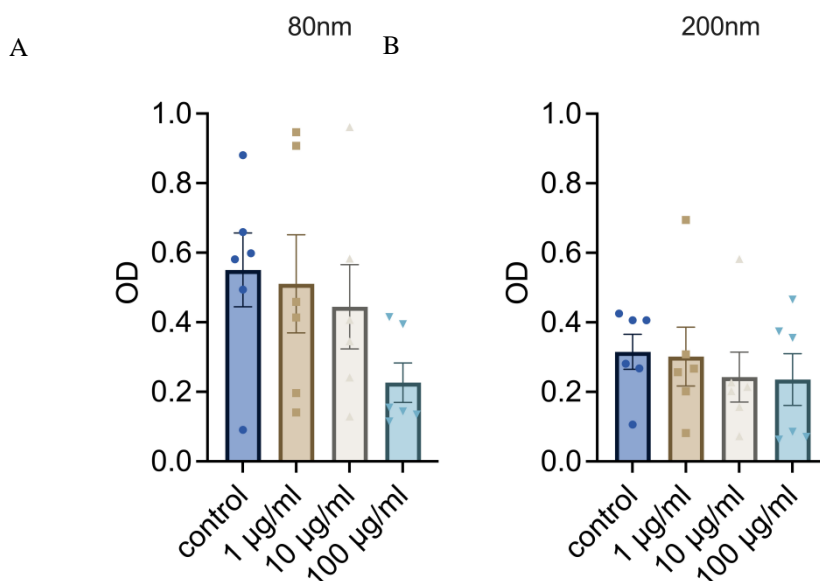
## 2.5 Cell Apoptosis by Flow Cytometry

In the cell apoptosis test, Annexin V-FITC as well as propidium iodide (PI) were used as fluorescent markers to indicate the degrees of cell apoptosis. Annexin V was a phospholipid-dependent protein which was capable of combining with the exposed phosphatidylserine (PS) on the cell membrane of the early apoptotic HCT116. FITC was a fluorescent molecule used to mark Annexin X. Propidium Iodide (PI) was a nucleic acid stain which could pass through late apoptotic cells to dye their nucleus. The combination of both molecules was used in the apoptotic test to distinguish different stages of HCT116.

Two 6-well plates seeded by HCT116 were used to test cell apoptosis. Each plate represented one size of PS microplastics in the later procedure when adding 1  $\mu$ g/mL of PS microplastics. Cell seeding process was the same as testing the cell cycle. After cultivation of HCT116 for 24 hours, all the superficial fluids of the complete culture medium were aspirated out from the 6-well plates. The already prepared chitosan solution was extracted by 1 mL into a new centrifuge tube and followed by 9 mL of culture medium. The chitosan solution was evenly mixed with culture medium. 2 mL of culture medium was added into two wells for both the 80 nm and 200 nm groups. One well was added by 1 mL of chitosan solution followed by 1 mL of culture medium (chitosan & culture medium). The last three wells with two of them added by 2 mL of PS microplastics and one of them added by 1 mL of PS microplastics with 1 mL of chitosan solution (plastics & chitosan). Chitosan was added to testify its capability of abating the cytotoxicity of PS microplastics and to examine whether single chitosan with culture medium would positively influence HCT116 or not. The 6-well plates were placed back into the incubator after adding microplastics separately in them. After cultivation for 24 hours, the supernatant was discarded from the 6-well plates and 2 mL of PBS was used to rinse the wells. The HCT116 in each well were extracted using 500  $\mu$ L of trypsin (without EDTA) and 1000  $\mu$ L of complete culture medium. The mixture was added into centrifuge tubes and centrifuged at 1000 rpm for 3 minutes. The process was repeated with 1 mL of PBS for two times, and the centrifugation values were adjusted to 2000 rpm for 5 minutes. 500  $\mu$ L of binding buffer, 5  $\mu$ L of Annexin V-FITC as well as 5  $\mu$ L of propidium iodide were added into each centrifuge tube with HCT116. The solutions were fully mixed and placed under aluminium foil (avoid light) for 10 minutes. Finally, the tubes were placed onto a flow cytometer and cell apoptosis was observed.

### 3. Results

#### 3.1 Comparison of HCT116 Absorption of Light



**Figure 3.** The bar charts showed the results of MTT (indicated by OD values) under 80 nm (A) and 200 nm (B) of PS microplastics groups.

The x-axis stood for PS microplastics concentration, and the y-axis stood for OD values to reflect absorption of light by HCT116. Different colors represented different concentration of PS microplastics (1 µg/mL, 10µg/mL, 100µg/mL).

The trends of absorption of light were clearly shown in the image and indicated by OD values. The results showed that the absorption of light (OD values) was not statistically significant in both the 80 nm and 200 nm groups (Figure 3).

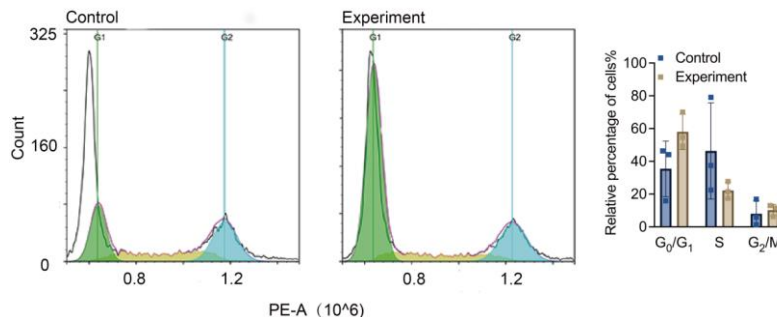
In the 80 nm group, the absorption of light of HCT116 gradually decreased as the concentration of PS microplastics increased from 1 µg/mL, 10 µg/mL to 100 µg/mL. The OD value was 0.57 when the concentration of 80 nm PS microplastics was 1 µg/mL and gradually decreased to 0.51 and 0.43 (corresponding to 10 µg/mL and 100 µg/mL of 80 nm PS microplastics concentration). There was a comparatively significant jump of OD values when the concentration of PS microplastics was soaring from 10 µg/mL to 100 µg/mL. The OD values shifted from 0.43 directly to 0.21 dramatically. Higher concentrations of 80 nm PS microplastics could potentially pose a more fatal threat to HCT116 according to the results.

In 200 nm group, there was no significant variation in both statistical analysis and the trend of OD values. Increasing concentration of PS microplastics from 1 µg/mL, 10 µg/mL to 100 µg/mL created subtle fluctuations in cell activity (indicated by OD values). The values of OD were around 0.5 with no significant differences. This phenomenon showed that 200 nm size of PS microplastics could not cause dramatic damages to HCT116 and this may be due to its large size and incapability of being taken into the intracellular space by endocytosis.

#### 3.2 HCT116 Cell Cycle Analysis

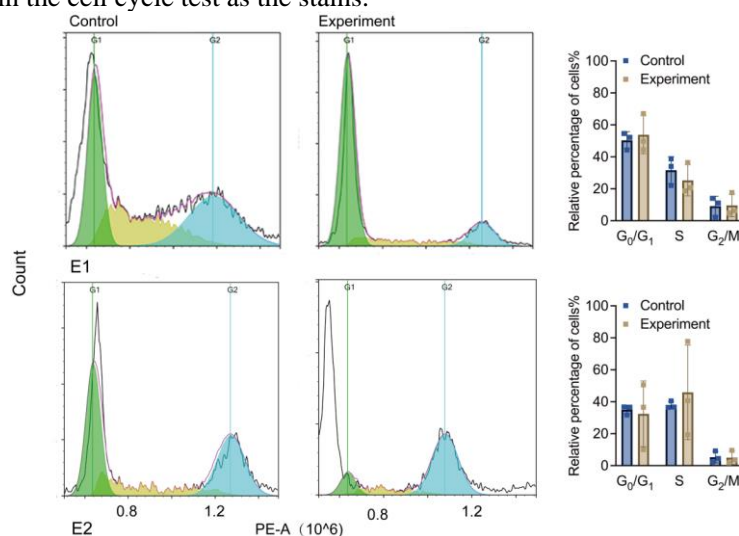
Flow cytometer was utilized in testing the cell cycle and the data derived from it clearly reflected the percentage of HCT116 in different phases of mitosis. The more specific division of phase into groups was based on the concepts that early interphase had G0, G1 and with S phase acted as an intermediate phase and finally G2 phase ended. In our study, G0 phase and G1 phase were integrated into a section and G2 as well as M (mitosis) phase belonged to a section. The cytotoxicity test of 80 nm PS microplastics group was carried out by a 6-well plate before testing the cell cycle. The upper three wells were control groups containing only culture medium and the downward three wells were added by 2mL of 1 µg/mL of 80 nm PS microplastics. The reason for this was that 10 µg/mL or 100 µg/mL

concentration of 80 nm PS microplastics would directly kill the HCT116 before testing them. This conclusion was based on the MTT test for cell activities as well as repetitive experiments in 6-well plates using 100  $\mu\text{g/mL}$  of 80 nm PS microplastics. All HCT116 were died under high volume as well as high concentration of 80 nm PS microplastics. The floating and aggregating white HCT116 on the surface of the solution was a remarkable characteristic of dead cells.



**Figure 4.** The graph represented the 80 nm PS microplastics experiment in cell cycle test and the experiment contained a control and an experimental group to make a comparison.

HCT116 in 80 nm PS group was exposed for 36 hours. The chart on the right showed the relative percentage of HCT116 in 3 different phases of mitosis in the control and experimental group at 80 nm PS group. RNase and propidium iodide was used in the cell cycle test as the stains.



**Figure 5.** The graph represented two repeating experiments of the 200 nm PS microplastics group in cell cycle test and the each experiment contained a control and an experimental group to make a comparison.

The top image showed the first experiment and was exposed under PS microplastics for 36 hours and the bottom image was the second experiment and only exposed for 15 hours. The chart on the right showed the relative percentage of HCT116 in 3 different phases of mitosis in the control and experimental group at 200 nm PS group. RNase and propidium iodide was used in the cell cycle test as the stains.

Figure 4 referred to the 80 nm PS microplastics group. The exposure duration was 36 hours under PS microplastics. It was palpable that the relative percentage of HCT116 in 80 nm PS microplastics solution was significantly higher in G<sub>0</sub>/G<sub>1</sub> period and lower in S phase than in the control group respectively. HCT116 had around 59% of cells stayed in G<sub>0</sub>/G<sub>1</sub> period and 22% of cells stayed in S phase in the 80 nm experimental group. The results displayed significant differences with the control group. The control group of HCT116 only possessed around 37% of cells in G<sub>0</sub>/G<sub>1</sub> phase but with 43% of cells in S phase which presented opposite effect with the experimental groups. 80 nm PS microplastics group greatly deterred HCT116 from entering or beginning mitosis because S phase was crucial in DNA replication of cells for subsequent mitosis process. Decreased amount of cells in that phase also indicated that less HCT116

cells were able to successfully complete cell division and make copies of themselves.

Figure 5 referred to 200 nm PS microplastics. There are two 6-well plates containing 200 nm PS microplastics and they were repeated for two different periods of time. The first experiment of HCT116 (Figure 5) cell cycle test exposed for the same time period- 36 hours under the 200 nm PS microplastics and the second group (Figure 5) in the bottom was only exposed for 15 hours. It was clear that short exposure period in the bottom showed slightly higher percentage of cells in S phase (42%) and relatively lower percentage of cells in G0/G1 period (31%) comparing with control groups and this seemed to be explained by the early compensatory response of HCT116 when initially exposed under 200 nm of PS microplastics and cells could possibly try to turn into S phase more rapidly in order to resist the effect of short time exposure of PS microplastics.

When the exposure time of HCT116 in 200 nm of PS microplastics was the same as the 80 nm PS microplastics group (36 hours) (Figure 5), HCT116 showed obvious increasing percentage of cells in G0/G1 phase (around 51%) and apparent decreasing percentage of cells in S phase (23%) compared with control groups. The trend was similar to that of the 80 nm PS group exposed under the same time period. The G2/M phase for both 80 nm PS and 200 nm PS group showed a subtle increase in G2/M phase but the variation was not significant when compared with control groups. The results demonstrated that both 80 nm and 200 nm of PS microplastics would restrain the completion of cell cycle and 80 nm PS microplastics displayed higher depression to HCT116 in cell cycle than that of 200 nm PS microplastics. Short exposure time would cause temporary increase in S phase and drop in G0/G1 phase and longer exposure time could lead to higher G0/G1 phase with shorter S phase which fatally damaged the cells.

### 3.3 Cell Apoptosis Analysis

Figure 6 and Figure 7 showed the results of HCT116 apoptosis and Figure 6 represented the 80 nm group and Figure 7 represented the 200 nm group. There were four sectors in each image and each single dot represented a cell. Q3-1 stood for cells which suffered from mechanical damage. Q3-2 stood for late apoptotic and necrotic cells. Q3-3 stood for living cells and finally Q3-4 stood for viable apoptotic cells.

In the experiments, 1  $\mu\text{g}/\text{mL}$  of both 80 nm and 200 nm PS microplastics were used in the 6-well plates for the purpose of making the cell treatment. For both the 80 nm and 200 nm groups, there were 2 wells acting as normal controls and were marked as NC (normal control) groups. Normal controls were added by 2 mL of culture medium. 1 well of the 6-well plate was added by 1 mL of chitosan solution followed by 1 mL of culture medium and was marked as CC (chitosan and culture medium) groups. There were two wells containing 2 mL of microplastics solution which were marked as P groups (PS microplastics solution). The last well in the 6-well plates was labeled as the PC group because it contained 1 mL of PS microplastics solution as well as 1 mL of chitosan solution. The CC group was compared with the NC group in order to make sure that the chitosan solution would not negatively influence the activity of HCT116 without PS microplastics solution. The PC group was compared with P groups and this was used to testify whether chitosan solution could abate the toxicity of PS microplastics solution and reduce the quantity of cell apoptosis.

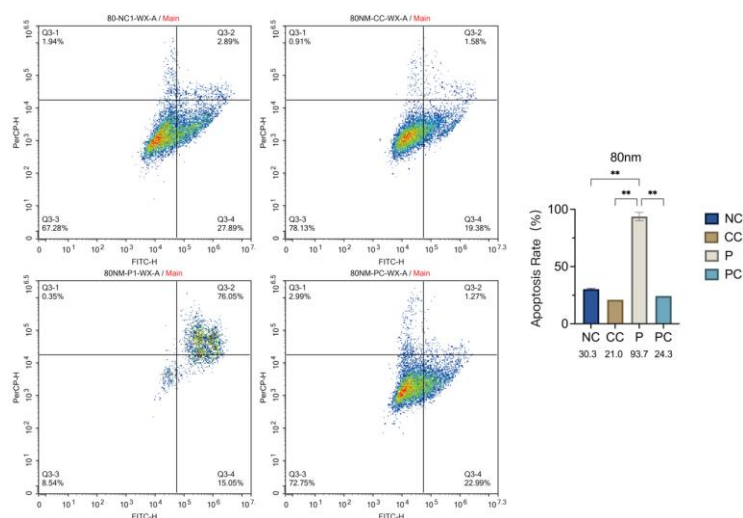
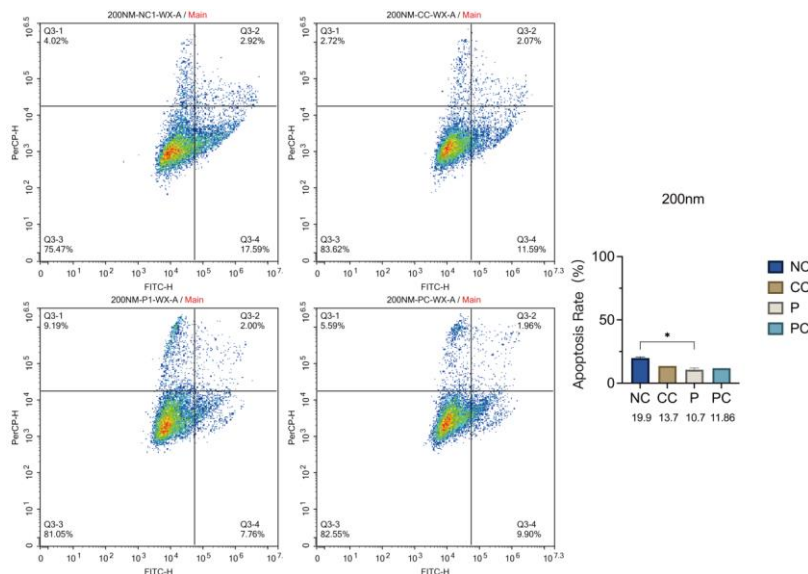


Figure 6. The graph represented 4 images of fluorescent intensity which corresponded to NC, CC, P and PC.

The bar chart showed the apoptosis rate of HCT116 in NC, CC, P and PC. 1  $\mu\text{g/mL}$  of 80 nm PS microplastics was used. In the cell apoptosis test, flow cytometer was used but with different stains (using Annexin V and propidium iodide) compared with that of the cell cycle test (using RNase and propidium iodide). PerCP-H in the graph (y-axis) represented the stain propidium iodide used in the experiment due to the default format of the flow cytometer and FITC (x-axis) stood for Annexin V. Axis and colors represented the intensity of cell samples in corresponding channels.



**Figure 7. The graph represented 4 images of fluorescent intensity which corresponded to NC, CC, P and PC. 1  $\mu\text{g/mL}$  of 200 nm PS microplastics was used.**

The bar chart showed the apoptosis rate of HCT116 in NC, CC, P and PC. In the cell apoptosis test, flow cytometer was used but with different stains (using Annexin V and propidium iodide) compared with that of the cell cycle test (using RNase and propidium iodide). PerCP-H in the graph (y-axis) represented the stain propidium iodide used in the experiment due to the default format of the flow cytometer and FITC (x-axis) was stood for Annexin V. Axis and colors represented the intensity of cell samples in corresponding channels.

In the 80 nm PS group, P group (containing PS microplastics) had the most highest apoptosis rate of HCT116 than the rest of the three groups, NC, CC and PC. The difference was statistically significant when compared with any of the left groups. In P group, the HCT116 apoptosis rate reached an astonishing 93.7% and then the rest of groups were closed to 20-30%, more specifically, 30.3%, 21.0% and 24.3%. The data confirmed the detrimental effects of 80 nm PS microplastics on HCT116 even in low concentration (1 $\mu\text{g/mL}$ ). 80 nm PS microplastics could severely deter normal cell activity and dramatically raise the quantity of cell death so it was necessary to notice them and to be vigilant about their fatal effects.

In the 200 nm PS group, the cell apoptosis rate was statistically significant when comparing the P group with NC group. The data was not significant when comparing the P group to the CC and PC groups. The overall trend showed that the percentage of cell apoptosis was not remarkable. The overall percentage of cell apoptosis was between 10% to 20%. More specifically, 19.9%, 13.7%, 10.7%, 11.86% were corresponded to NC, CC, P and PC. After excluding randomness, it could be concluded that 200 nm of PS microplastics had little impact on HCT116. The exposure of HCT116 under 1  $\mu\text{g/mL}$  of 200 nm PS microplastics solution would not cause significant damage to cells or even lead to abnormal programmed cell death.

### 3.4 Chitosan Reduced Toxicity of PS

In both Figure 6 and Figure 7, the results conform to our previous assumption that chitosan could abate the toxicity of PS microplastics. The apoptosis rate of HCT116 dropped dramatically after adding the chitosan solution when comparing the P to PC groups and the apoptosis rate of PC was even lower than in the NC groups. The results also proved that chitosan would slightly decrease the cell apoptosis rate even without adding PS microplastics. This meant that chitosan would not produce a negative effect on HCT116 without adding PS and it could slightly extend the life

span of HCT116.

In the 80 nm PS microplastics group (Figure 6), P group had a cell apoptosis rate of 93.7 % and the PC group had an amazing 24.3 %. The comparison of the apoptosis rate of HCT116 between P and PC was statistically significant. The CC group had a cell apoptosis rate of 21.0% and it was relatively lower than the NC group with a cell apoptosis rate at 30.3%.

In the 200 nm PS microplastics group, P groups had a cell apoptosis rate of 10.7% and PC groups had an apoptosis rate of 11.86%. The apoptosis rate was close. The difference was not as significant as the 80 nm group, but the ability of chitosan to resist HCT116 apoptosis could still be proved by compared NC (19.9%) with PC (11.86%) groups. P groups had no significant difference comparing with CC and PC groups. According to the results, 200 nm of PS microplastics had a relatively lower negative impact on HCT116 which corresponded to the trend in both MTT and cell cycle test. The valuable role played by chitosan was crucial in the test.

#### 4. Discussion

In contemporary society, with the rapid development of industries and factories, more products containing microplastics are spreading among the world. Microplastics can enter the human body through plenty of ways such as oral exposure, inhalation or even skin exposure. They are potentially jeopardizing the health of humans in different levels although the detrimental effects haven't become visible in a short period of time. Hazards of disease may have incubation periods which are difficult to discovered [9]. As a result, more research will be focusing on the detriments of various microplastics as well as possible materials or chemicals which possess the potential in the future to reduce these hazards. The study we have done and relative research demonstrated the importance of knowing the actual hazards produced by microplastics and the pathway as well as mechanisms microplastics could act on.

In our study which mainly focused on PS microplastics, we discovered the strong toxicity of the 80 nm size of PS microplastics in MTT, cell cycle as well as cell apoptosis test. It was also interesting that 200 nm size of PS microplastics possessed significantly lower toxicity to HCT116 than 80 nm size. In 200 nm PS microplastics solution, most HCT116 functioned just as normal cells with normal cell apoptosis and absorption of light. PS microplastics would normally carry negative charges in the aqueous environment to cover some of the original hydrophobic characteristics to blend into its environment. Besides, PS microplastics could also form Van der Waals interaction with other partially charged particles because it had net zeta potentials [10]. In our experiment, PS microplastics were placed in the culture medium to have cell intoxication test. Under the exposure of light, as well as actions by reactive oxygen species (ROS) in the culture medium, originally highly exposed PS microplastics would probably undergo oxidation and form more -COOH as well as -OH groups on the surface. It was also common for PS microplastics to carry some functional groups which could release hydrogen ions and become negatively charged particles when they were manufactured. More H<sup>+</sup> ions would also dissociate into the solution when the surrounding aqueous environment became more alkaline and were left with high density charged functional groups. This condition was reached when culture medium and nutrients were present. This feature was important for the interaction of PS microplastics and HCT116 in the solution.

For the 80 nm size of PS microplastics, the size fitted with the most common pathway of endocytosis by HCT116 including the Clathrin-mediated endocytosis (CME). The CME pathway of endocytosis relies on clathrin (formed by triskelion) to form vesicles (requires ATP) which specifically fit on the 80 nm size of PS microplastics. The interaction of the surface of 80 nm PS microplastics to the plasma membrane or more specifically the transmembrane receptors of HCT116 would likely trigger the pathway of coating, scission as well as uncoating to bring the vesicles containing PS microplastics into the endosome-lysosome system. The vesicles will therefore be carried toward the lysosome for further processing. Normal pathogens or infinitesimal particles would swiftly be destroyed by the lysosome and its various hydrolytic enzymes. However, PS microplastics was considered non-biodegradable and was difficult to be broken down. This would cause the accumulation of PS microplastics in the lysosome. As previously mentioned, some PS microplastics would carry charges which were likely to attract hydrogen ions or other charged particles inside the lysosome, so the internal PH environment of the lysosome would become unbalanced due to the abnormal function of V-ATPase (a type of proton pump directly using hydrolysis with no phosphorylation process).

Additionally, the uneven surface of PS microplastics would possibly punch holes on the membrane of lysosome. This could also destruct the integrity and permeability of lysosome's surface membrane which also known as lysosomal membrane damage (LMD). The damage of the lysosome would lead to more harm to other organelles inside the cells. More destructive enzymes as well as ROS were released into the cytoplasm. The ROS would attack the

nucleus and cause the DNA in the chromosome to suffer irreparable damages. This directly activated nucleases and triggered the cell apoptosis pathway which led to programmed cell death. The successive reaction like ferroptosis would take place [10]. Besides, ROS and destructive enzymes could also deter the normal function of the endoplasmic reticulum as well as working of mitochondria. Increasing quantity of misfolding proteins occurred which led to cell death. For the damaged mitochondria, apoptosis signals could also be initiated by activating Ced-4 and Ced-3 to start the subsequent pathway of programmed cell death [11]. These possible results caused by PS microplastics could explain the graph of cell apoptosis test with an extremely high rate of cell apoptosis rate under the exposure of 80 nm size PS microplastics.

For 200 nm size PS microplastics, the size was too large to initiate endocytosis. The microplastics in the solution would just be blocked outside the cell instead of being directly taken in. The colossal size of PS microplastics would be impossible to be surrounded by the plasma membrane of HCT116 and transported to various organelles like the lysosome. The intracellular damage was therefore less significant. For the larger size of PS microplastics, the particles would have a lower diffusion rate due to greater mass, and they were likely to aggregate together as they were charged. The overall distance between the PS microplastics as well as HCT116 in the bottom of the disks would be larger although larger PS microplastics could possibly sink to the bottom and contact adherent HCT116. Therefore, the overall negative effects of 200 nm PS microplastics would be lower than 80 nm microplastics. The difference of the cell apoptosis rate between the culture medium group to the microplastics group would be similar.

The detoxication capability of chitosan was demonstrated in an apoptosis test using flow cytometry. There was evidence in recent research which showed the ability of chitosan in removing microplastics from guts like polyethylene [12] as well as the cellulose of plant tissues [1]. Possible mechanisms were also proposed. When chitosan entered the stomach, it would dissolve in gastric juice and become a positively charged dietary fibre. This ability assists the chitosan in removing microplastics from the intestine and block its entry into intestinal cells [13]. The dissociation of chitosan into positive charged particles would likely happen with the interaction of PS microplastics in the culture medium [1]. Positively charged chitosan bound to negatively charged ethyl cellulose and aggregation took place as well. In our study, the negatively charged PS microplastics would probably combine with the positively charged chitosan in the culture medium and prevent both 80 nm as well as 200 nm of PS microplastics from interacting with HCT116 [14]. Less HCT116 would inaccurately recognize PS microplastics as a substance that could be taken in. The endocytosis rate would therefore greatly decrease so PS microplastics had a lower toxicity on HCT116. Some reports showed that chitosan would also act physically instead of having a direct chemical reaction [15]. However, the actual mechanisms of action of chitosan still required more experiments and research to explore.

## 5. Conclusion

This study demonstrates the size- and concentration-dependent cytotoxicity of PS microplastics on intestinal cells and identifies chitosan as an effective mitigating agent. These results contribute to the understanding of microplastic toxicity mechanisms and offer potential strategies for reducing their health impacts.

In this study, the co-culture model as well as the oxidative stress mechanisms between the PS microplastics and HCT116 emphasizes the hazards and hidden dangers caused by these microplastics. The process of experiments and tests objectively presented more evidence to the current society and humans about the hidden dangers around their living environment (dangers are referred to as PS microplastics here). The utilization of chitosan in the experiments also provided us with more ideas and methods of finding possible materials or chemicals which could possess the characteristics of abating the negative impacts from microplastics. In our study, the influences of PS microplastics to intestinal cells were discovered. Chitosan acting as an interesting material was also used to discover specifically its effects on PS microplastics, and this attempt provided more references for future research to discover more effective drugs as well as materials to maintain intestinal health [16]. The research also endowed the medical realm with a platform to create new therapeutic interventions and called on people to avoid potential impacts caused by microplastics in the long run.

## References

- [1] Liu D, Shimizu M. Ingesting chitosan can promote excretion of microplastics. *Sci Rep.* 2025;15:14041. <https://doi.org/10.1038/s41598-025-96393-w>
- [2] Jing S, Wang Y, Chen Y, et al. Standardizing pyrolysis gas chromatography mass spectrometry for nanoplastics and microplastics

- detection to advance environmental research. *npj Emerg Contam.* 2025;1:2. <https://doi.org/10.1038/s44454-025-00001-5>
- [3] Zha H, Li S, Zhuge A, Shen J, Yao Y, Chang K, Li L. Hazard assessment of airborne and foodborne biodegradable polyhydroxyalkanoates microplastics and non-biodegradable polypropylene microplastics. *Environ Int.* 2025;196:109311. <https://doi.org/10.1016/j.envint.2025.109311>
- [4] Zhang Y, Men J, Yin K, et al. Activation of gut metabolite ACSL4/LPCAT3 by microplastics in drinking water mediates ferroptosis via gut–kidney axis. *Commun Biol.* 2025;8:211. <https://doi.org/10.1038/s42003-025-07641-8>
- [5] Park K-M, Kim B, Woo W, Kim LK, Hyun Y-M. Polystyrene microplastics induce activation and cell death of neutrophils through strong adherence and engulfment. *J Hazard Mater.* 2024;480:136100. <https://doi.org/10.1016/j.jhazmat.2024.136100>
- [6] Zhao B, Liu R, Guo S, et al. Large-sized polystyrene microplastics induce oxidative stress in AML12 cells. *Sci Rep.* 2025;15:26616. <https://doi.org/10.1038/s41598-025-11577-8>
- [7] Wang F, Zhang Q, Cui J, Bao B, Deng X, Liu L, Guo M-y. Polystyrene microplastics induce endoplasmic reticulum stress, apoptosis and inflammation by disrupting the gut microbiota in carp intestines. *Environ Pollut.* 2023;323:121233. <https://doi.org/10.1016/j.envpol.2023.121233>
- [8] Zhang X, Wang J, Liu Y, Wang H, Li B, Li Q, Wang Y, Zong Y, Wang J, Meng Q, Wu S, Hao R, Li X, Chen R, Chen H. In situ profiling reveals spatially metabolic injury in the initiation of polystyrene nanoplastic-derived intestinal epithelial injury in mice. *Sci Total Environ.* 2024;927:172037. <https://doi.org/10.1016/j.scitotenv.2024.172037>
- [9] Rashid E, Hussain SM, Ali S, et al. Polystyrene microplastics exposure in freshwater fish, *Labeo rohita*: evaluation of physiology and histopathology. *Sci Rep.* 2025;15:12888. <https://doi.org/10.1038/s41598-025-95811-3>
- [10] Manabe S, Haga Y, Tsujino H, et al. Treatment of polyethylene microplastics degraded by ultraviolet light irradiation causes lysosome-deregulated cell death. *Sci Rep.* 2024;14:24008. <https://doi.org/10.1038/s41598-024-74800-y>
- [11] Hwang J, Choi D, Han S, et al. Potential toxicity of polystyrene microplastic particles. *Sci Rep.* 2020;10:7391. <https://doi.org/10.1038/s41598-020-64464-9>
- [12] Prasetyo S, Santos CA, Sugih AK, Kristianto H. Utilization of chitosan as a natural coagulant for polyethylene microplastic removal. *Sustain Chem Environ.* 2025;9:100225. <https://doi.org/10.1016/j.scenv.2025.100225>
- [13] Ortiz C, Müller L, Borges L, de Almeida Pinto LA, Cadaval TRS, Tesser MB, Pedrosa VF, Romano LA, Wasielesky W, Ventura-Lima J. The use of chitosan as an antioxidant in the feed of cultivated *P. vannamei* shrimp against oxidative stress induced by exposure to microplastics. *Mar Environ Res.* 2024;202:106747. <https://doi.org/10.1016/j.marenvres.2024.106747>
- [14] Ullah H, Chang H, Safi NA, Somia B, Wang J, Qiao A, Ahmad M, Nasrullah AR, Su R. Advances in chitin and chitosan-based materials for microplastics treatment. *Carbohydr Polym.* 2025;368(Part 1):124073. <https://doi.org/10.1016/j.carbpol.2025.124073>
- [15] Zhang YC, Thomas Y, Kim E, Payne GF. pH- and voltage-responsive chitosan hydrogel through covalent cross-linking with catechol. *J Phys Chem B.* 2012;116(5):1579–85. <https://doi.org/10.1021/jp210043w>
- [16] Herrera-Vázquez SE, Elizalde-Velázquez GA, Gómez-Oliván LM, Chanona-Pérez JJ, Hernández-Varela JD, Hernández-Díaz M, García-Medina S, Orozco-Hernández JM, Colín-García K. Ecotoxicological evaluation of chitosan biopolymer films particles in adult zebrafish (*Danio rerio*): a comparative study with polystyrene microplastics. *Sci Total Environ.* 2024;929:172757. <https://doi.org/10.1016/j.scitotenv.2024.172757>