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# *The Toxic Effects of Polystyrene Microplastics on Colonic Epithelial NCM460 Cells*

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***Abstract:*** Lots of recent studies focused on the impact of microplastics on human health and investigates the location of it inside the body. Most of the study aiming to orient the traces of polystyrene microplastic use a kind which pigments were added inside making it easier to be observed. But weather the treate particles could replace the original one? The following study was set to investigate the impact of original or marked polystyrene microplastic (PS MPs) on NCM460 cells and how the pigments within could have impact on them. Cells experienced a bath of different concentration(100μg/mL, 200μg/mL, 400μg/mL) of two different formulas including PS and colored PS. Cell Counting Kit - 8 (CCK - 8) assay was used to detect the cell viability. The result shows that, first, PS MPs do have an negative impact on the and the viability. On top of it, pigments also has significant impact on the result tested by the colored ones. CCK data decreased while ROS increased illustrating that oxidative stresses could also be triggered by PS MPs and the colored one. In a word, PS MPs do have an impact on human cells and colored ones increases the damage, as a result, colored ones shouldn't be seen as the substitute for the original PS MPs. This work provides theoretical foundations for the investigation of distribution within scientific studies.

## 1. Introduction

As we all know, plastic is one of the most convenient materials we can use in our daily lives. It is lightweight, can withstand a certain degree of high temperature, and is not easily corroded [1]. As such an outstanding item, it's as well broadly used in different ranges like making bottles or bags, even tables, etc [2]. After the usage, waste can't be avoided, contaminating different environments (soil, ocean, forest and so on) this gives a great challenge to the animals and other lives in the eco system [3]. Microplastics are defined as tiny pieces of plastics sized in a range 1μm and 1 mm of which that are scattered out in the environment [4][5]. polystyrene (PS), as one of the main types of plastics, exists in a great amount within the environment. Current studies showed that PS microplastic (MPs) has a great impact on human organs and cells in lots of aspects, including reduce of its vitality, triggering inflammations, oxidative stress triggers, apoptosis promotion, and even causing intestinal barrier dysfunction [6,7,8,9]. Firstly, lots of studies showed that PS will reduce the vitality of gut cells. When the microplastics immerge into intestinal cells, they would interrupt the metabolic process of

them. PS MPs could also activate related inflammation, because cells would recognize them as intruders and secrete more inflammatory signaling pathways, such as nuclear factor κB, this would furtherly rise the expression of inflammation-related genes to increase. As a result, the body exposed to microplastic would end up with chronic diseases.

The toxicity of nano-scale plastics in microplastics may be stronger, not only the toxic effects of microplastics, but also the toxicity of nanomaterials. It's really important to look into how nanoplastics, especially PS particles, can affect us. These tiny particles can get into our bodies through eating, breathing, or even touching our skin. Cells react differently to these particles depending on their type, size, and surface changes. For example, amino-modified particles seem to be more harmful than those without modifications or with carboxyl groups [10]. Zhang et al. Focused on at the respiratory system but what they discovered about how nanoplastics enter cells and affect them was also useful for gut research [11]. Using A549 and BEAS-2B cell lines, they showed that nanoplastics can cause oxidative stress and mess up cell function. This gives us a model that might work for studying intestinal cells too. It shows we need a better way to mimic real human exposure conditions to confirm these findings. Teng et al. did something different by looking at how PS nanoparticles affect zebrafish using targeted metabolomics and microbiome sequencing [12]. Their study found that exposure to these nanoparticles can change neurotransmitter metabolism, cause gut inflammation, and disrupt microbial communities. This impacts both gut health and overall well-being, suggesting that the effects could even pass down through generations. Putting all this together, there are some big questions we need to answer in this field. The impact of nanoplastic was given lots of attention. Due to the high attention of people on the investigation of PS MPs and its impact on human gut. It is meaningful to do such further investigation on the further impact of the pigments within, and whether it can replace original pigments, because lots of people directly use the colored ones to investigate the toxicity and location of PS MPs. And whether it can replace original pigments. We need standardized methods and exposure conditions to make studies more comparable. Differences in experimental design, like particle concentration and exposure time, make it hard to evaluate nanoplastic toxicity. In short, these researches reviewed here highlights the challenges and opportunities in understanding how PS nanoplastics affect gut cells and overall health. Therefore, our work aimed to investigate how PS and colored PS MPs affect intestinal cells. And the interaction of pigments.

## 2. Materials and Methods

### 2.1 Experimental Materials

The CCK-8 and ROS kit were purchased from syndicate Beyotime company (Shanghai, China). PS (Figure 1) and colored PS brought by Macklin company (Shanghai, China). DMEM medium was obtained from Gibco (USA). Fetal bovine serum (FBS) was purchased from Ehcell (Suzhou, China). Penicillin - streptomycin - amphotericin B in cell culture medium purchased from Biosharp (Hefei, China). NCM460cells bought from ACCA (USA).

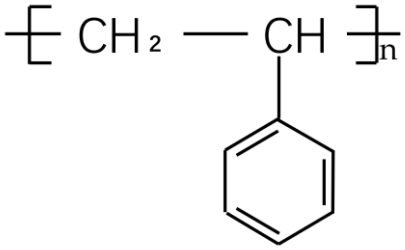


Figure 1. The chemical structure of PS microplastic.

## 2.2 Cell Culture

NCM460 cells (ACCA) were cultured in substrate containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were preserved in a humidified incubator at 37 °C with 5% $CO_2$. When the cell density reached 80%-90%, they experience another term of culture. The second cultivation process involved washing the cells with phosphate - buffered saline (PBS), basing them with 0.25% trypsin - EDTA for 2 - 3 min, and made the detached cells grow in the fresh medium.

## 2.3 CCK - 8 Assay for Cell Viability Detection

The principle of the CCK-8 assay is based on the reduction of WST-8 (a tetrazolium salt) by mitochondrial dehydrogenases in cells to form a formazan product. Briefly, NCM460 cells were planted into 96-well plate at a density of $2 \times 10^3$ cells per well were supposed to be able to attached on the bottom of the plate overnight. Then, the cells were treated with 3 agentia of PS MPs (0, 100, 200, and 400 μg/mL, respectively) for 2 h and 24h. After this process, 10 μL of CCK - 8 liquor was added to each well, and the plates were preserved at 37 ℃ for 1 h. Then the plates were placed in a microplate reader to determine. The cell viability was calculated as follows: Cell viability (%) = (Absorbance of treated group / Absorbance of control group) × 100%.

## 2.4 Data Analysis

All experiments were performed in triplicate. The data were performed in the mean ± standard deviation (SD). GraphPad prism software was used for statistical analysis. Two - way analysis of variance (ANOVA) followed by Tukey's post - hoc test was used to discuss the differences between groups. When the value was satisfied in $p < 0.05$ it would be considered statistically significant.

# 3. Results and discussion

## 3.1 Effect of PS Microplastics on the Viability of NCM460 Cells

To investigate how PS and colored PS MPs affect intestinal cells, we proceeded an experiment to investigate the viability of the cells after being placed into the microplastic. As a result, shown in Figure 2A, the viability of NCM460 cells declined significantly after being placed into a concentration of PS microplastic. After 24 h of treatment, compared with the control group, the cell viability began to decline when the concentration of PS reached only 100μg/mL. After 2h of treatment, the implosions of the restriction of the microplastic on cell viability was more obvious. At the highest concentration of PS MPs (400 μg/mL) after 2 h of treatment, the cell viability was only 75.08 % of the control group. The decrease in cell viability induced by PS may be attributed to several factors. Firstly, MPs can damage cell membranes, affecting their structure and function [13]. The water-repelling surface of polystyrene microplastics can disrupt the lipid bilayer, increasing permeability and causing cellular leaks [14]. Secondly, PSs would be considered as a kind of intruder to the cells and as a result force inflammation to happen within the cells, from which part of the decrease of the vitality comes from.

With the curiosity to the metabolic capability of the cells (NCM460) to polystyrene microplastics, we continued and proceeded another experiment, in which we placed the microplastic with the cells under a condition of 37 degrees and 5% CO2 for 24 hours, that by adding CCK-8 and again placing it under the previous condition for 1 more hour, finally ending it by testing the OD of at the depth of 450nm. We got a result.

As shown in Figure 2B, surprisingly we found that most of the numbers comparing to the control

group were not significant. What's more, with the growth of the concentration of the polystyrene formula, the cell viability also appears to be seen in a growing tendency. To discover why the tendency was continuously increasing, we checked more about the characteristics of micro polystyrene particles. And we found that these particles were able to absorb to the proteins in the cells. In this case we can assume that the microplastic were all trapped inside the dead body of the cells which they killed and absorbed on to the proteins they secreted while they are still alive. This also explained why cells died within 2 h. By the way because it was appearing to be in an increasing tendency also got its own explanation.

Although, most of the comparation between colored groups and the group without color appeared to be not significant but it could be able to be discovered that, cells in the colored group were preserved in a way of which their vitality were lower comparing to the group which pigments were not contained within the MPs. To explain this phenomenon, I found that most artificial pigments could cause similar effects as PSs on gut cells by triggering inflammation and it's also inductive of oxidative stress by increasing the generation of reactive oxygen species leading to so. This could damage the membrane lipids and the DNA of cells as well as weakening the structures a function. On top of it, it could also lead to the death of intestinal epithelial cells by activating intracellular apoptotic signaling pathways, such as mitochondrial pathway. So colored ones should not be considered to replace original PS MPs under certain conditions.

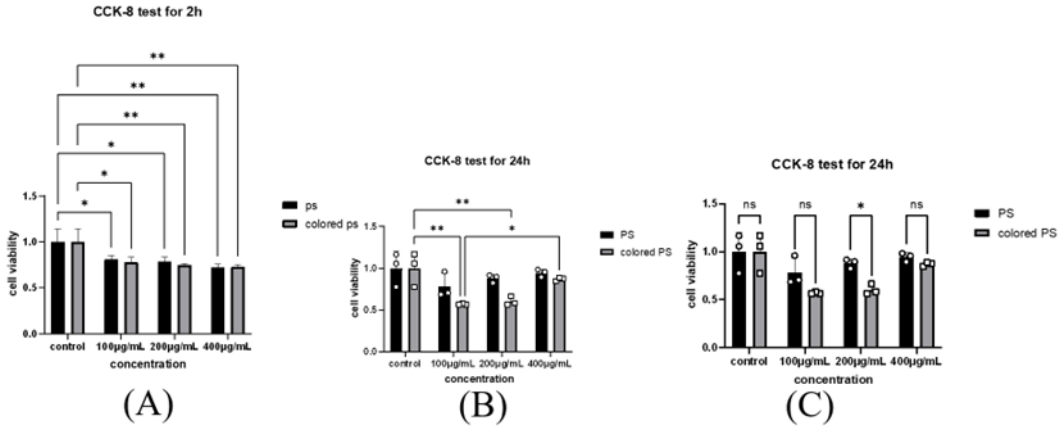


Figure 2. Cell viability of NCM460 cells treated with different concentrations of PSs and colored PS for 2h (A) and 24h (B and C). *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$ compared with another group.

## 4. Conclusion

In conclusion, this study illustrates that Polystyrene micro plastics da have significant impacts on NCM460 cell viability, reduces its growth, moreover, the pigments within the colored PS MPs increases such damage, by the way suggests that colored ones shouldn't be used for experiments, to make a promise on the accuracy of the data observed from which. But micro plastics also do have negative impacts on NCM460 cells and the forbidden of plastic trash should be reduced. But I admit that this study does have some limitations. First, we only investigated the impact of PS MPs on NCM460 cells, but other cells maybe would have different references on these specific particles. Second, PS MPs could not represent all the microplastics but we only used the PSs for investigation. Future study could be carried out in order to investigate the influence of such things.

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