MEDS Basic Medicine (2025)
Clausius Scientific Press, Canada

DOI: 10.23977/medbm.2025.030108
ISSN 2616-2091 Vol. 3 Num. 1

Study on the Toxic Effects of Nanoplastics on Colonic Epithelial NCM460 Cells

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Keywords: Polystyrene microplastics; human colorectal mucosal cells; cytotoxicity CCK - 8

Abstract: This study delved deeply into the effects of PS - 100 and NH_2 - PS - 100 on NCM460 cells, filling a void in the research regarding the impacts of these two specific microplastics on this type of cell within the realm of microplastic cytotoxicity. It laid the groundwork for the subsequent establishment of a more comprehensive theoretical framework of microplastic cytotoxicity. This study aimed to explore the effects of 100 - nanometer polystyrene microplastics (PS - 100) and 100 - nanometer amino - functionalized polystyrene microplastics (NH_2 - PS - 100) on normal human colorectal mucosal cells (NCM460 cells). NCM460 cells were treated with different concentrations of PS - 100, NH_2 - PS - 100, and their mixtures, and the cell viability was detected using the Cell Counting Kit - 8 (CCK - 8) assay. The results showed that the co - exposure of PS - 100 and NH_2 - PS - 100 could significantly reduce the viability of NCM460 cells in a concentration - and time - dependent manner. This finding provides a theoretical basis for understanding the potential health risks of microplastic.

1. Introduction

In today's era, plastic has become one of the most commonly used and versatile materials in our daily lives. To a large extent, this is due to its high stability, versatility, and ease of production. However, its widespread use has led to the generation of a large amount of plastic waste. The tiny fragments resulting from the decomposition of plastic waste give rise to microplastics and nanoplastics, which is one of the ways secondary microplastics are produced. They are widely present in the environment, as well as in drinking water and food. Primary microplastics come from artificially produced plastic pellets.

Polystyrene (PS) is widely used in the catering and service industries and is one of the most common components of microplastics in the ocean. On the one hand, plastic materials are lightweight, corrosion - resistant, and have a wide range of applications, which can make life more convenient and improve people's quality of life. On the other hand, when plastics enter the environment and food, we find that we are facing a global challenge. Although the production of plastic products has been curbed, the global production of plastic products is still increasing year by year. In recent years, the global annual production of plastic products has continued to grow. In 2023, the total global production of plastics reached 413.8 million tons, among which China's total plastic production

accounted for 33.3%. If the global plastic demand trend continues, it is estimated that the global annual plastic production will exceed 1.1 billion tons by 2050. Most plastic products are disposable and can only be discarded after use, remaining in the environment as plastic pollution. After microplastics enter the environment, they can enter the human body through the food chain, causing damage to the human body. The negative effects of microplastics may be related to the chemical reactions that occur during the degradation of microplastics and the additives present in plastics[1-3]. Microplastics in the human intestine mainly come from the ingestion of food containing microplastics. In recent years, the health damage of microplastics to humans has received much attention[4,5].

Therefore, our work was to conduct an in vitro experiment to study the toxic effects of microplastics on the human intestine. We will explore the effects of 100nm PS microspheres and 100nm amino - PS microspheres on human intestinal cells respectively, as well as the effects of a mixture of PS-100 and NH_2 - PS - 100 on human intestinal cells.

2. Materials and Methods

2.1 Experimental Materials

Materials used in this experiment were human normal colorectal mucosal cells (NCM460 cell), 10% fetal bovine serum (FBS), 1% Penicillin/Streptomycin, Dulbecco's Modified Eagle's Medium (DMEM), phosphate buffered saline (PBS) solution, and trypsin. PS (Figure 1) microspheres was purchased from Macklin company, with a diameter of 100 nm, at a concentration of 2.5% w/v, in a volume of 1 mL, labeled as PS - 100. Amino - functionalized PS (Figure 2) microspheres, provided by Macklin China. The concentration was 2.5% w/v, the volume was 1 mL, and the particle size was 100 nm. It has been labeled as NH_2 - PS - 100.

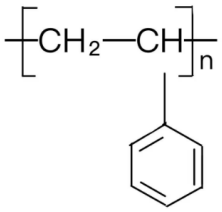

Figure 1. The chemical structure of PS microplastic.

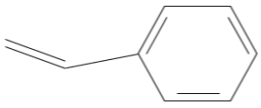

Figure 2. The chemical structure of NH_2 - PS - 100 microplastic.

2.2 Cell Culture

NCM460 cells are cultured in DMEM medium containing 10% FBS and 1% penicillin/streptomycin at 37°C with 5% CO_2 humidity. PBS solution and trypsin are also needed.

First, 10 mL of the prepared culture medium was placed into a new sterile petri dish. Then, the

cells were rinsed with 2 mL of PBS. Subsequently, 1 mL of trypsin solution was added to initiate enzymatic digestion. After 2 min of digestion at 37 ℃, the enzymatic reaction was terminated by adding 2 mL of fresh culture medium. The detached cells were collected by washing the culture surface, and the resulting cell suspension was transferred to a centrifuge tube. Centrifugation was performed at 1200 rpm for 3 min to collect the cells. The supernatant was carefully decanted. And 1 mL of fresh culture medium was added to resuspend the cells, creating a homogeneous cell suspension. Finally, the cells were transferred to the pre-prepared petri dish and cultured in a humidified 5% CO2 incubator at 37 ℃.

2.3 Cytotoxicity Assay

First, the subculture cells were counted and seeded into a 96-well plate at a density of 2×10^6 cells/mL. After 24 h incubation, different samples (PS microspheres, amino PS microspheres, and a mixture of PS microspheres and amino PS microspheres prepared in DMEM without FBS) at concentrations of 100μg/mL, 200μg/mL, and 400μg/mL were respectively added to each well and cultured for 2 h or 24 h. Then the supernatant was carefully removed. And 10 μL of CCK8 solution was added to each well and culture for 1 hour. Another group was set up, which was cultured for 24 hours under the same conditions except for the culture time.

3. Results and discussion

3.1 Cytotoxicity of PS - 100 and NH2 - PS – 100 on NCM460 cells

Based on the Figure 3A, in the group cultured for 2 hours, there was no statistical significance the PS - 100 group, the NH_2 - PS - 100 group, and the PS - 100 + NH_2 - PS - 100 group compared with the control group. No obvious toxic effects of microplastics were observed within 2 hours.

Based on the Figure 3B, in the group cultured for 24 hours, statistical analysis showed no significant differences between the PS - 100 group, the NH_2 - PS - 100 group and the control group. In the PS - 100 and NH - PS - 100 co - exposure group, only the 400 μg/mL co - exposure group had statistical significance ($p < 0.01$). According to the results, we found that there were no toxic effects when they were alone, with no significant changes in terms of time or dosage. However, when in low - concentration mixtures, there was no toxicity, while high - concentration mixtures were toxic. The mechanism of the combined toxic effect on intestinal cell damage may interact with intestinal cells through the following pathways, leading to toxic effects.it was speculated that adding PS microspheres (PS - 100) or amino - polystyrene microspheres (NH2 - PS - 100) alone did not exert significant toxicity on cells. However, a mixture of PS - 100 and NH_2 - PS - 100 can be toxic to cells ($p < 0.01$). It is thus speculated that the cell proliferation in the PS - 100 group of the 2 - hour group might be due to the short culture time, during which PS - 100 has not yet exerted significant toxicity, along with the normal proliferation of the cells themselves.

As shown in the Figure 3, the co - exposure of PS - 100 and NH2 - PS - 100 exhibited stronger toxicity. It was speculated that this may be due to the superposition of their toxicities. At the same time, there is no obvious toxicity in the 2 - hour group, but there is in the 24 - hour group. Therefore, we found that short - term exposure to these two types of microplastics does not result in toxicity. However, when the exposure time is longer, or the concentration is higher, or in the case of combined exposure, toxic effects may occur. It is speculated that this may be because the cultivation time of the 2 - hour group is too short, and the drugs have not yet exerted their effective effects.

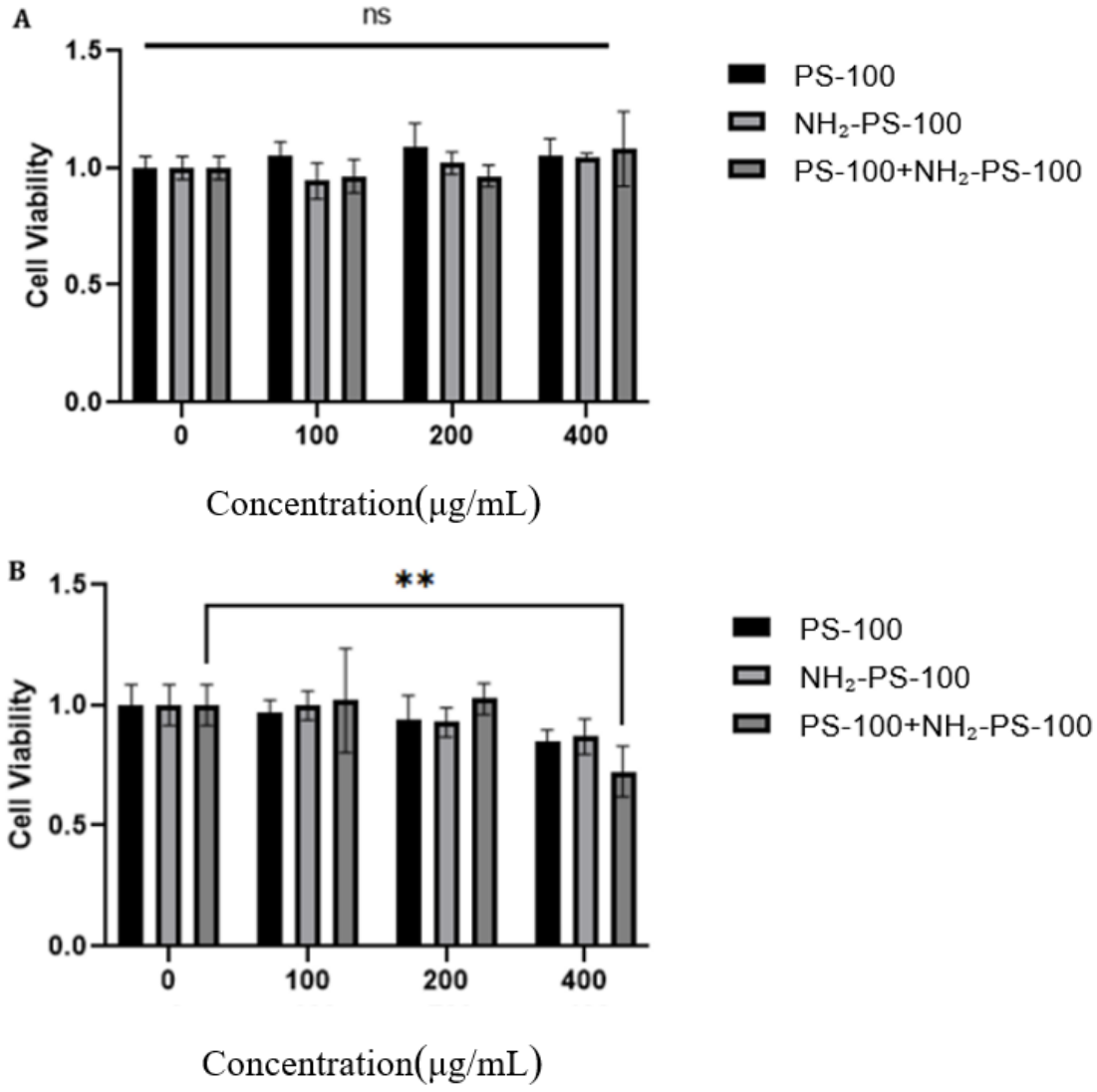

Figure 3. The PS - 100 and NH_2 - PS - 100, and their co - exposure led to cytotoxicity in NCM460 cells. (A) NCM460 cells were exposed to microplastics 2 hours. (B) NCM460 cells were exposed to microplastics 24 hours. $^{**}p < 0.01$ compared with control group.

4. Conclusion

This study demonstrates that the mixture of high - concentration polystyrene microplastics and amino - functionalized polystyrene microplastics can significantly reduce the viability of normal human colorectal mucosal cells. Understanding the potential effects of PS - 100 and NH_2 - PS - 100 on human normal colorectal mucosal cells is of great significance for assessing the health risks of microplastics. However, effects of microplastic remain to be further investigated.

This study has certain limitations. Firstly, only in vitro studies were conducted. The differences in the in vivo environment can affect the results and cannot fully reflect the in vivo situation. In future research, it is necessary to further prove the toxicity effects of PS - 100 and NH_2 - PS - 100 on colorectal mucosal cells through animal models. Secondly, the potential molecular mechanisms, such as the oxidative reactions occurring in the body and the activation of related specific signaling pathways, have not been deeply studied. Future research could focus on exploring the reaction

mechanisms to better understand the toxicity mechanisms of microplastic.

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