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Toxicity of micromilimeter of polyvinly chloride and polystyrene sulfonic acid microplastics toward NCM460 cells

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Abstract: To investigate the difference in toxicity between polyvinyl chloride (PVC) and polystyrene sulfonic acid (PS), this study tests the toxicity of PVC and PS on NCM460 cells. NCM460 cells are located in the human colonic epithelium. By testing these two different microplastics at various concentrations and exposure times (e.g., 2 hours and 24 hours), the CCK-8 assay results show that PS is a safer material for intestinal cells than PVC. This work provides theoretical and experimental evidence for evaluating the risk of microplastics to intestinal cells.

1. Introduction

In modern life, a wide variety of plastic products bring convenience to people. Common plastic polymers, such as polyethylene terephthalate, polypropylene, polyvinyl chloride (PVC), polystyrene sulfonic (PS), and polyethylene, exist widely in daily life for various forms like straws in the beverage, packaging cover and plastic bottle [1]. Although plastic act an indispensable role with people's lives, however people lack the awareness of plastics and their potential danger. Polyvinyl chloride which is widely used in the fields of construction, electronic devices, medical care, packaging, and daily necessities, will gradually degrade into nanoplastics in the environment, especially in the ocean. After being ingested by microorganisms and aquatic organisms, it will eventually return to the human body through the food chain. For instance, Rakesh Kumar found that microplastics can trigger cell inflammation, DNA damage, gene toxicity, and the development of cancer and tumors. Moreover, existing research had found that microplastics with less than 0.025 μm can have a negative impact on the human immune system, metabolism, etc, and trigger the cell inflammation [2].

In previous research, people have found that microplastic may triggered human health through step-to-step approach. Microplastic which contain in food, drinking water, make up and factory can induce the cell, gene and nervous system’s toxicity or even cancer in different parts of the body [3]. From early experiment, researchers had tested the toxicity of polystyrene on soil and plant, impact of nanoplastic to human health and polyvinly chloride of medical material’s cell toxicity examine [4]. They had tested it in different temperature and time. Researchers found that it’s important to test polyvinly chloride because it contributes 28% in plastic market consuming [5]. However, what people

found was some spacious. Therefore, these studies didn't bring remarkable relation or result to real life, which hadn't enough value to the society. They had shown the effect of the plastic but didn't present it in different concentration. Because in the real-world situation, there are many different concentrations of plastic that can be absorbed in human body, so it will be closer to the real-world case if the concentration will be presented. There had been relatively a little amount of studies on the cytotoxicity and oxidative damage of specific types of microplastics to colonic epithelial cells.

So, this work focused on two common plastics, polystyrene sulfonic acid (PS) and polyvinyl chloride (PVC), which to explore their cytotoxicity to NCM460 cells of colonic epithelial tissue [6]. This study will fill up some gaps in this field and provide more reliable data and theoretical support to the understanding of the health risks of microplastics. In my hypothesis, I assumed that the toxicity of polyvinly chloride is lower than polystyrene sulfonic acid. Because nowadays the use of polyvinly chloride is been used wider in the real world, therefore the related department would know it's toxicity, so this material is been used, on the contrary the use of polystyrene sulfonic acid is relatively lower than the previous material, so theoretically its toxicity is higher than polyvinly chloride. The contribution of this study will help to add more research findings in this area of studying, and it can provide much more information and knowledge for the society, so they will avoid it on purpose. Also, this experiment can give as a significant information to manufacturing department, so they will change the national production standard according to the research. It can promote the development of the new type environmental pollutant and the research of microplastics, but also make up the deficiency of this area of studying.

2. Material and methods

2.1 Experimental material

Both particles were spherical shape. Cell counting kit - 8 (CCK - 8) was getting from Beyotime company. Polyvinyl chloride (PVC) (Figure 1) purchased from Aladdin company and polystyrene sulfonic acid (PS) (Figure 2) purchased from Macklin Company.

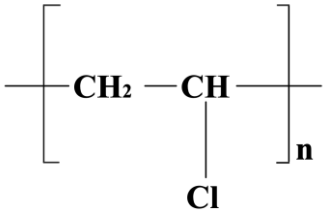


Figure 1. The chemical structure of PVC.

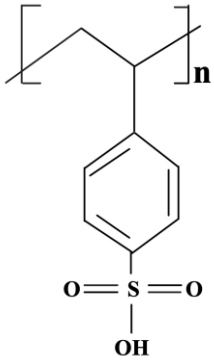


Figure 2. The chemical structure of PS.

2.2 Cell culture

The process of culturing NCM460 cells (ACCA) involved growing them in DMEM (Gibco, USA) supplemented with three different types of antibiotics and 10% fetal bovine serum (FBS) (Gibco, USA). The cells were maintained in an incubator at 37 ℃ with 5% humidity. Once the cell density reached 80-90%, they were passaged to a new plate to ensure optimal growth conditions for subsequent experiments. For experimental procedures, the cells were seeded in 96-well plates (U.S. corning brand) at a density of 2×10^3cells per well and incubated for 24 h to allow attachment and stabilization before treatment with different concentrations of microplastics. This culturing method was crucial for maintaining cell viability and ensuring accurate assessment of the cytotoxic effects of polyvinyl chloride (PVC) and polystyrene sulfonic acid (PS) microplastics using the CCK-8 assay.

2.3 CCK-8 assay

The CCK-8 assay was employed to systematically examine and quantify the viability and metabolic activity of the NCM460 cells following exposure to different concentrations of microplastics. The cells were precisely plated in 96-well plates (U.S. corning brand) at a density of 2×10^3cells per well to ensure optimal growth and uniform distribution. The plates were then incubated at 37 ℃ for 24 hours to allow the cells to adhere to the wells and stabilize, creating a consistent baseline for the experiment. After the incubation period, the cells were treated with samples of different concentrations (0, 100, 200, 400 μg/mL) of both polyvinyl chloride (PVC) and polystyrene sulfonic acid (PS) microplastics for two different exposure times: 2 hours and 24 hours. This experimental design aimed to simulate varying real-world exposure scenarios and assess the acute and chronic effects of microplastic exposure on intestinal cells. Following the treatment periods, 10 μL of CCK-8 solution was carefully added to each well and the plates were incubated again at 37 ℃ for 1 hour to allow the cells to interact with the CCK-8 reagent, which produces a colorimetric response proportional to the number of viable cells. Finally, the absorbance at 450 nm was accurately determined using a microplate reader, providing quantitative data on cell viability that was further analyzed to compare the toxic effects of different microplastic types and concentrations on NCM460 cells.

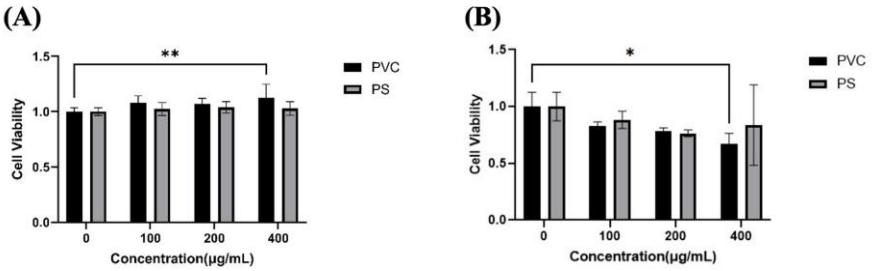
2.4 Data analysis

All experiments were meticulously conducted three times to ensure the reliability and reproducibility of the results. For statistical analysis, the powerful and widely-used software GraphPad Prism was employed. To compare the differences between various groups, a comprehensive two-way analysis of variance (ANOVA) was applied. This statistical method allowed for the simultaneous assessment of the effects of two independent variables—microplastic concentration and exposure time—on cell viability. Following the ANOVA, Tukey's post hoc test was conducted to determine specific significant differences between individual group means. The significance level was set at $p < 0.05$, meaning that any observed difference with a p-value below this threshold would be considered statistically significant. This rigorous statistical approach ensured that the conclusions drawn from the experimental data were robust and valid, providing a solid foundation for the interpretation of the microplastics' cytotoxic effects on NCM460 cells.

3. Result and discussion

3.1 Effect of PVC and PS on the viability of NCM460 cells

To investigate the toxicity differences between polyvinyl chloride (PVC) and polystyrene sulfonic acid (PS) microplastics on NCM460 cells, a series of experiments were conducted with exposure times of 2 hours and 24 hours. As depicted in Figure 3A, 2 hours after the addition of the samples, no significant differences in cell viability were observed among most groups, except for the group treated with 400 μg/mL PVC, which exhibited significant cell proliferation activity ($p < 0.01$). This unexpected proliferation might be attributed to the cells being in a specific growth phase at the time of treatment, where the microplastics did not exert significant toxic effects. However, when incubated for 24 hours, the group treated with 400 μg/mL PVC displayed significant cytotoxicity ($p < 0.05$), while all concentrations of PS showed no significant toxic effects (Figure 3B). This indicates that the toxic effects of PVC on NCM460 cells are concentration and time-dependent, whereas PS appears to be less toxic under the same experimental conditions.



*$p < 0.05$, **$p < 0.01$, *$p < 0.001$ compared with the control group.

Figure 3. Cell viability of NCM460 cells after being treated with different concentrations of PVC and PS for (A) 2 h and (B) 24 h.

4. Conclusion

To conclude, this experiment has demonstrated that microplastics can have a toxicity impact on NCM460 cells and decrease their viability. The damage was found to be significant at certain concentrations when tested over different time periods. Specifically, both PVC and PS showed non-toxicity at relatively low concentrations. However, PVC exhibited toxicity toward NCM460 cells at a concentration of 400 μg/mL. Therefore, it can be inferred that these two microplastics may cause negative effects on human colonic epithelium. Exploring the health risks posed by microplastics to the human colonic site is crucial for understanding their potential impact on human health.

However, this work has some limitations that should be considered for future research. First, the human colonic site is a complex environment with diverse cells and microplastics. To scientifically assess health risks, experiments should mimic the real environment of the human colon. This means that the combined effect of different microplastics should be investigated, as they might cause more significant toxic effects when present together. Second, the current results may not accurately reflect real-world situations. To obtain more precise outcomes, further research should focus on specific population groups or cell types. Despite these limitations, this work provides certain evidence for the study of microplastic toxicity and serves as a foundation for future research in this area.

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