Surface Charges of Polystyrene Nanoplastics Affect Their Distribution in Mice

Baimanov D.[1], Song Z.D.[1,2], Wang L.M.[1], Yuan Q.X.[3], Zhang Z.Y.[1,2]

[1]CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China

[2]School of Nuclear Science and Technology, University of the Chinese Academy of Sciences, Beijing 100049, China

[3]Beijing Synchrotron Radiation Facility, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China

*corresponding author: Zhang Zhiyong
e-mail: zhangzhy@ihep.ac.cn

Abstract Micro- and nanoplastics can enter the human body through ingestion, inhalation, and skin contact, and their distribution within the body and impact on health have attracted widespread attention. This study aimed to examine how particle size and surface charge influence the biodistribution of polystyrene nanoplastics (PS NPs) in mice following intravenous administration. Our results indicate that the majority of fluorescent PS NPs, regardless of their charge or size, predominantly accumulated in the liver, which is pivotal for filtration. Notably, negatively charged COOH-PS NPs exhibited significant accumulation in the lungs 30 min post-injection, in contrast to positively charged NH_2-NPs. Specifically, 100 nm COOH-PS NPs demonstrated marked lung accumulation, implying that these anionic particles can more effectively traverse the vascular endothelial barrier. Subsequently, we analyzed the protein corona composition on the surface of nanoplastic particles and discovered vitronectin and fibrinogen as crucial plasma proteins interacting with negatively charged PS NPs. This corona facilitates integrin αIIbβ3 receptor-mediated phagocytosis by lung endothelial cells, accounting for the localization of PS NPs in the lungs. These findings underscore the significance of considering both surface charge and protein corona composition in assessing the safety of nanoplastics.
Keywords: polystyrene nanoplastics, protein corona, magnetic isolation, lung accumulation, integrin receptors

1. Introduction

Nanoplastics, mainly from single-use plastic waste, are increasingly entering human bloodstreams, posing significant public health concerns. Polystyrene nanoparticles, often found in food packaging and foam, have been detected in about 36% of blood samples (Leslie, Van Velzen et al. 2022). However, their biological identity and health effects, especially surface modifications, remain poorly understood. While the liver is the primary site of nanomaterial accumulation after intravenous injection due to its role as a biological filtration system, nanoplastics can also reach the lungs through various pathways (Kim, Eygeris et al. 2024). When nanoplastics enter the bloodstream, plasma proteins rapidly form coatings, determining their biological identities and driving their circulation to the heart, lungs, and other major organs. This process, mediated by the plasma protein corona, influences cell and tissue recognition, leading to specific organ accumulation patterns.

This study delves into how corona proteins affect the biodistribution and lung accumulation of PS NPs. We proposed an integrated methodology using magnetic isolation and high-resolution imaging. Our findings provide valuable insights into the lung accumulation pathway of nanoplastics and their health implications, helping future risk assessment.

2. Methods

Carboxyl- and amine-functionalized iron-core PS NPs (Fe_3O_4@SiO_2@PS) sized 100 nm and 300 nm were synthesized using a solvothermal reaction. Fluorescent PS NPs with average diameters of 50 nm, 100 nm, and 240 nm, functionalized with carboxyl and amine groups, were prepared by dissolving KPS and SDS in water, adding St and rhodamine B, and oscillating for 24 h.

Iron-core PS NPs were incubated with 10% human plasma in PBS bufferat 37 °C for 30 min. After protein corona formation, the complexes were washed and separated using a magnet. Protein corona composition were analyzed by LC-MS/MS. Purified proteins were also analyzed by SDS-PAGE and CD spectrophotometry.

Female C57BL/6J mice were administered fluorescent or iron-core PS NPs via intravenous injection. The organs from mice treated with fluorescent PS NPs were imaged using an IVIS in vivo imaging system. Lung samples from mice treated with iron-core PS NPs underwent synchrotron radiation μ-CT imaging.

3. Results and Discussion

To tackle the complexity of determining nanoplastic distribution in the bloodstream and assessing potential human hazards, we incorporated an iron-core within the polymer shell of nanoplastics. This approach achieved two goals. First, it enabled high-throughput and fast protein corona isolation via magnetic separation, overcoming centrifugation limitations where traditional methods fail to distinguish nanoplastics from biomolecules due to similar sizes and densities. Second, it allowed high- resolution, 3D *in vivo* visualization of nanoplastic distribution through μ-CT. The study investigated the protein corona of iron-core PS NPs of sizes 100 and 300 nm with surface modifications of -COOH and -NH_2 groups. Results showed that vitronectin levels in the protein corona were significantly influenced by NP size and surface modification. Larger NPs tended to have higher vitronectin levels, possibly due to increased surface area providing more binding sites. However, fibrinogen levels were higher in smaller PS NPs. These proteins play crucial roles in mediating cellular adhesion, migration, tissue repair, and influencing NP aggregation and immune response. We hypothesize that elevated levels of vitronectin and fibrinogen enhance the binding of PS NPs to integrin receptors in lung tissues, facilitating their selective accumulation and cellular internalization.

To investigate the accumulation of PS NPs in organs, particularly the lungs, we conducted *in vivo* experiments on C57BL/6J mice. Results showed that after intravenous administration, the majority of PS NPs initially accumulated in the liver. However, negatively charged COOH-NPs exhibited significant accumulation in the lung 30 min post-injection, unlike positively charged NH_2-NPs. Notably, 100 nm COOH-NPs showed significant lung accumulation. Synchrotron radiation techniques were used to visualize the distribution of 100 nm iron-core PS NPs, confirming the lung accumulation pattern. COOH-NPs accumulated along the lung lumen structures, whereas NH_2-NPs did not follow this pattern. The consistency between *in vitro* corona fingerprints and *in vivo* accumulation results suggests that the corona composition plays a crucial role in directing tissue-specific accumulation.

To determine the role of the protein corona in NP recognition, we focused on vitronectin, the most abundant protein. Circular dichroism results indicated slight changes in vitronectin's secondary structure after binding to COOH-NPs. *In vitro* uptake experiments showed that less conformational change in vitronectin led to higher uptake of NH_2-NPs compared to COOH-NPs. We found that vitronectin mediates integrin receptor-dependent uptake by HUVEC cells. Fibrinogen, another abundant component in the corona, also contributes to the recognition of COOH-NPs by integrin receptors in endothelial cells. The higher abundance of fibrinogen on COOH-NPs may contribute to their greater enrichment in the lung.

To determine the distribution of 100 nm PS NPs within the lungs post-treatment, flow cytometry was utilized. Results demonstrated that COOH-NPs accumulated at significantly higher levels in lung tissue compared to NH_2-NPs. This pattern was observed across various lung cell types, including endothelial cells, monocytes, and neutrophils, with COOH-NPs showing greater internalization. We focused on lung endothelial cells as they are the first to interact with nanoparticles after crossing the bloodstream's endothelial barrier. Immunofluorescence experiments further supported these findings, showing colocalization of PS NPs with CD41/CD61 receptors on endothelial cells, suggesting that vitronectin mediates the accumulation of COOH-NPs in lung endothelial cells via integrin receptors.

4. Conclusions

This study offers vital insights into the *in vivo* behavior and fate mechanisms of nanoplastics, as well as the health risks linked to nanoplastic pollutants. These findings will inform future development and use of safer plastic materials.

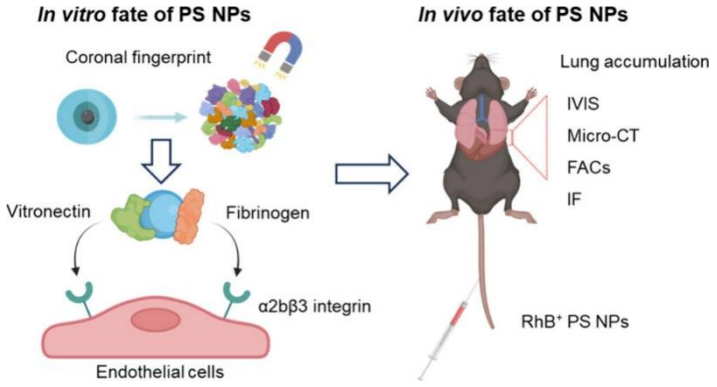

Figure 1. *In vitro* and *in vivo* fate of polystyrene nanoplastics

References

Kim, J., Y. Eygeris, R. C. Ryals, A. Jozić and G. Sahay (2024). Strategies for non-viral vectors targeting organs beyond the liver. *Nature Nanotechnology* **19**(4): 428-447.

Leslie, H. A., M. J. Van Velzen, S. H. Brandsma, A. D. Vethaak, J. J. Garcia-Vallejo and M. H. Lamoree (2022). Discovery and quantification of plastic particle pollution in human blood. *Environment international* **163**: 107199.