

# Polystyrene nanoplastics: Surface- and Size-dependent effects on human primary endothelial cells

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As of 2015, humans had produced 8.3 billion metric tons of plastics, 6.3 billion tons of which had already become waste. Although plastic wastes are considered long-lasting and stable in the environment, under the influence of different physical and chemical factors plastics undergo fragmentation into micro- and nanometer-level particles, named micro- (100 nm – 5 mm) and nanoplastics ( $\leq$  100 nm) (MNPLs), respectively. During this weathering process, plastics suffer from changes in their physicochemical surface properties that can influence its toxicological potential. Nowadays, there is increasing evidence suggesting that environmental MNPLs can reach the human body through different pathways: ingestion, inhalation and dermal contact. Indeed, Leslie et al., (2021) have been able to detect plastics  $\geq$ 700 nm, including polystyrene, in whole blood samples representative of the general population. However, the potential effects of MNPLs on the health of exposed individuals are still unknown and require further research.

In the present study, polystyrene nanoparticles (nPS) and Human Umbilical Vein Endothelial Cells (HUVECs) were used to better understand what are the toxicokinetic and toxicodynamic interactions of MNPLs with the vascular system.

To explore the influence of physicochemical properties on the observed effects, representative nPS of different sizes (PS-COOH 30 nm, 50 nm, and 100 nm) and surface characteristics (Pristine PS, carboxyl (-COOH) and amino (-NH<sub>2</sub>)) were included in the study.

Our results suggest that although all nPS are internalized by HUVECs, the internalization dynamics are modulated based on the functionalization and the size of the particle. Interestingly, our flow cytometry data also shows that both PS-COOH 50 nm and PS-COOH 100 nm are able to modify the morphology of the cell and increase its inner complexity/granularity. When analyzing possible toxic effects by treating the cells with a concentration of 100  $\mu$ g/mL we observe that only PS-NH<sub>2</sub> 50 nm is able to reduce cell viability (- 40% vs control; 12 h treatment). Finally, our first approach to study ROS generation when treating the cells with 100  $\mu$ g/mL of PS-COOH 50 and PS-COOH 100 nm for 24 h shows an increase in ROS production with both types of nPS particle (+ 70% in both cases vs control).

Overall, our results indicate a surface- and size- dependent effect of nPS on HUVEC cells. Further experiments are needed to fully understand the impact of MNPLs on the vascular system, and how the particle's properties influence the effects.

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