

Size-dependent effects of polystyrene nanoplastics on human primary endothelial cell function

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Micro- and nanoplastics (MNPLs) have emerged as pervasive environmental pollutants, raising growing concerns about their potential health risks. Their documented presence in the human bloodstream highlights the need to understand their interactions with endothelial cells and their role in cardiovascular diseases such as atherosclerosis. However, the full implications of these interactions remain largely unknown. To address this, we investigated the impact of three sizes (30, 50, and 100 nm) of polystyrene carboxylated nanoplastics (PS-C-NPLs) on human umbilical vein endothelial cells (HUVECs) at 100 µg/mL concentrations. By integrating *in vitro* assays with bioinformatic transcriptomic analyses, we employed a multimodal approach to comprehensively investigate the effects of PS-C-NPLs on endothelial cells. *In vitro* assessments included nanoplastics (NPLs) internalisation (flow cytometry, confocal microscopy, TEM), morphological and internal complexity changes (flow cytometry), genotoxicity (comet assay), and functional alterations, such as cholesterol biosynthesis (Filipin III staining), migration (wound healing assay), angiogenesis, and inflammation (IL-6 ELISA). Additionally, RNA sequencing provided transcriptomic insights into the cellular response to PS-C-NPLs, complementing the *in vitro* findings and revealing molecular pathways underlying the observed effects. All three PS-C-NPL sizes were rapidly internalised by HUVECs within 20 minutes, inducing significant alterations in cell morphology, internal complexity, and function. PS-C-NPLs caused genotoxic damage and disrupted cholesterol metabolism, migration, angiogenesis, and inflammatory responses. Notably, some effects exhibited a size-dependent trend, with similarities emerging between carboxylated polystyrene (PS-C) 50 and 100 nm NPLs, while the smallest 30 nm NPL showed slightly distinct responses. Transcriptomic analyses reinforced these findings, revealing shared pathways across all three PS-C-NPLs — linked to cholesterol metabolism, endothelial-to-mesenchymal transition, DNA damage, and inflammation — alongside size-specific molecular signatures. This study is the first to comprehensively link transcriptomic changes to size-dependent functional alterations in endothelial cells induced by PS-C-NPLs. Our findings demonstrate that PS-C-NPLs significantly impair endothelial cell function and integrity in a size-dependent manner, underscoring their potential cardiovascular risks.

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