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Potential effects of in vitro digestion on the physicochemical and biological characteristics of polystyrene nanoplastics

**M. Morataya-Reyes^{1*}, L. Vela^{1,2}, A. Villacorta^{1,3}, T. Venus⁴, S. Pastor¹,
I. Estrela-Lopis⁴, R. Marcos¹, A. Hernández¹**

*¹Group of Mutagenesis, Department of Genetics and Microbiology,
Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain*

*²Facultad de Ciencias de la Salud, Universidad UTE,
Avenida Occidental y Mariana de Jesús, Quito, Ecuador*

³Facultad de Recursos Naturales Renovables, Universidad Arturo Prat, Iquique, Chile

*⁴Institute of Medical Physics and Biophysics, University of Leipzig, 04107 Leipzig,
Germany*

**herlem.morataya@uab.cat*

In humans, the main exposure route to micro/nano plastics MNPLs is via ingestion. To effectively determine the toxicological profile of MNPLs, the role of digestion must be considered. Accordingly, this study aims to determine the influence of the in vitro digestive process on the toxicity of polystyrene nanoplastics (PSNPLs) in three different human leukocytic cell lines. Using transmission electron microscopy (TEM) and scanning electron microscopy (SEM) we have determined that all particles are spherically shaped, with similar appearance and sizes but, the digested particles show a relevant tendency to agglomerate. The hydrodynamic radius shows that digested particles have a larger hydrodynamic size, and the polydispersity index (PDI) indicates that the non-digested particles are more monodisperse. These results agree with the results of the Z-potential showing that the digested particles have less Z-potential. Also, the particles were analyzed by Nano tracking analysis (NTA) to measure the hydrodynamic radius supporting the previously obtained data. Cell uptake was evaluated with the fluorescent polystyrene counterparts (fPSNPLs and dfPSNPLs) by flow cytometry and confocal microscopy. Results show that the three selected cell lines internalize more dfPSNPLs than fPSNPLs. When cell viability was assessed, only moderate effects were observed at the highest concentration of dPSNPLs in TK6 at exposures lasting for 24/48 h. No intracellular ROS production was observed in any of the cell lines at 24/48 h and, finally, genotoxic damage induction was detected only at 24 h exposure in THP1 cells, and at the highest concentrations. No oxidative DNA damage was detected at any time and in any cell line. Finally, the visualization and characterization of proteins on the surface of PS particles was done by Raman spectroscopy showing the association of proteins on the particle surface and information about the secondary protein structure on particle surface.

Funding:

This work was partially supported by the EU Horizon 2020 programme (965196, PlasticHeal), the Spanish Ministry of Science and Innovation (PID2020-116789, RB-C43), the Generalitat de Catalunya (2021-SGR-00731), and the ICREA-Academia programme to AH.

Keywords:

Nanoplastics, Polystyrene, digestion, cell uptake, cytotoxicity.